AUSTRALIAN EGG CORPORATION LIMITED

The importance of nests for the welfare of laying hens

A report for the Australian Egg Corporation Limited

by Greg M.Cronin & John L. Barnett

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Foreword

Housing laying hens in cages is a controversial animal welfare issue in the community, with impacts on public acceptance of current industry practices and the potential to lead to imposed future changes which may be associated with increased capital costs (e.g. compulsory use of modified cage designs such as 'furnished' cages). In a previous AECL- / DPI- co-funded project (Barnett and Cronin, 2005), the commercial Victorsson Trivselburen 8-bird Furnished Cage (Sweden), and the components of the cage furniture viz. nest box, dust bath and perch, were evaluated to determine the effects of the cage furniture on bird welfare, behaviour and egg production. The project showed that when present the furniture was well utilised, However, with the exception of the perch improving bone strength, cage furniture provided no quantifiable welfare benefits compared to hens in cages without furniture. Another finding was that in cages containing a nest box, 62% of eggs were laid in the nest box. Thus, about one-third of eggs from hens with access to a nest box were laid outside the nest boxon the wire cage floor. Many questions are therefore raised in relation to nest boxes for hens, and a major issue in the welfare debate for the egg industry concerns whether nest boxes are important to hen welfare.

This project focused on hens' choice of egg laying site in modern cages and the relationship between egg laying site and stress physiology as a measure of bird welfare. In the first experiment, methods for video recording hens in cages (including during periods of low light intensity) inside nest boxes and in total darkness, were developed and applied. In addition, a method for marking hens under infra-red light was developed to facilitate identification of hens from their group mates on the video record. Thus it was possible to record continuously the lives of the 112 hens in the experiment on digital video. From the video records the hens' pattern of egg laying (time and location) and consistency of laying in the nest box were determined. The video technology also enabled the 'capture' specific eggs and identification of the hens that laid the eggs. Measurement of stress hormones, either from blood samples or from egg albumen, to relate the behavioural characteristics to each hen's stress response, was also achieved. This experiment showed a consistent choice by individual hens for laying eggs either in the nest box or on the wire floor; this consistency of site selection provided a model whereby for the first time, the impact of nest site manipulation on stress physiology (and behaviour) could be determined in other experiments. The second experiment examined the timing of egg laying and the stress response of hens under 2 light-dark schedules. At completion of the main observations, the impact of blocking the nest box's entrance on stress responses of birds that were consistent nest box layers were investigated. Finally, the third experiment was a series of 3 smaller experiments where the effects of some features of the nest box (light and entrance h8) and social effects, on nest box use by hens were investigated.

This project was funded by industry revenue, which is matched by funds provided by the Federal Government, and by funds from the Department of Primary Industries, Victoria.

This report is an addition to AECL's range of research publications and forms part of our Research & Development program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

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About the Authors

Dr Greg Cronin joined the then Department of Agriculture (Victoria) in 1971, completed his MAgrSc. (University of Melbourne) in 1982 under the supervision of Drs Paul Hemsworth and Rolf Beilharz, and a PhD in 1985 at the Agricultural University of Wageningen, The Netherlands, under the supervision of Professor Piet Wiepkema. The theme of the PhD was the development of stereotyped behaviour in tethered sows, and implications for sow productivity of the performance of the behaviours. Since completing his PhD studies Greg Cronin has been employed as a research scientist/senior research scientist involved in research programs on animal behaviour, production and welfare at the Department of Primary Industries, Victoria, Werribee Centre. In June 2008 Greg was appointed Lecturer in Animal Behaviour and Animal Welfare Science, Faculty of Veterinary Science, University of Sydney. Dr Cronin remains a member of the Animal Welfare Science Centre, which is a joint Centre of the Department of Primary Industries, Victoria, University of Melbourne (Institute of Land and Food Resources) and Monash University (Departments of Psychology and Physiology).

Dr John Barnett completed his undergraduate degree in the UK (BSc Hons) and then completed a PhD at Monash University, in 1973. He subsequently held a post-doctoral position in the UK and a teaching/research position at La Trobe University, before joining the Department of Agriculture (Victoria) in the late 1970s. His research career spans 35 years and he has applied his area of expertise in stress physiology and its application to environmental physiology of mammals and birds to animal welfare. He has published over 150 papers in refereed scientific journals and chapters in books, plus over 300 additional publications including addresses to conferences, reports to funding bodies and extension articles. John is a member of the Animal Welfare Science Centre, and following his retirement from DPI Victoria in January 2008, he has been appointed Research Fellow at the Faculty of Land and Food Resources at the University of Melbourne. John is the program leader of the welfare program for the Australian Poultry Cooperative Research Centre.

Post-script
Associate Professor John Barnett and his wife Jenny Barnett tragically lost their lives in the Victorian bush fires on February 7th 2009. John was an internationally-respected animal welfare scientist, who made a significant contribution to the welfare of commercial poultry, and farm animals in general. John studied zoology in the United Kingdom after which he undertook post-graduate studies in Australia on the physiological and endocrine responses of birds and mammals to stressors. He was a strong advocate of a multidisciplinary approach to the scientific study of animal welfare. Throughout his career, John applied his broad scientific knowledge and skills to improve our understanding of farm animal welfare and developed new methodologies to study animal welfare. John was a keen science collaborator and communicator, with a strong commitment to building inclusive discussions between the different stakeholder groups interested in poultry welfare. His contribution to the scientific and technical literature on poultry welfare is outstanding.

Contents

List of Tables

List of Figures

List of Photographs

Abbreviations

ACTH	Adrenocorticotrophic hormone (a hormone from the pituitary gland that stimulates the synthesis and release of corticosterone from the adrenal glands)
AWSC	Animal Welfare Science Centre (a joint Centre of the Department of Primary Industries Victoria, University of Melbourne and Monash University)
AECL	Australian Egg Corporation Limited
C	Centre
CI	Confidence interval
cm	Centimetre
DPI	Department of Primary Industries, Victoria
g	Gram
EDTA	Ethylenediaminetetraacetic acid
EU	European Union
FL	Far left
H, h	Hour
HPA axis	Hypothalamic-pituitary-adrenal axis
IR	Infra-red light
IU	International Units
LED	Light emitting device
LH	Luteinizing hormone
min	Minute
m	Metre
mL	Millilitre
ML	Mid left
mm	Millimetre
NB	Nest box
ng	Nanograms
REML	Restricted maximum likelihood (model used for statistical analysis)
SD	Standard deviation
Std Dev	Standard deviation
SED	Standard error of difference between mean values

1 Executive Summary

This project focused on the relationship laying hens had with the nest box in their cage, and physiological measures of hen welfare, in 4 experiments that examined the presence or absence of a nest box, group size and light schedules.

1.1 Major findings

A unique feature of the methodology in this project was the use of low-light video and infra-red light, whereby each bird was uniquely identified and easily viewed on digital video to determine when and where each egg was laid.

Most hens chose a consistent site for egg laying by about the tenth egg. Over 6 experiments, about 2-thirds of hens consistently laid in the nest box and about 27% consistently laid on the wire floor (consistent was defined as at least 80% of eggs at the one site). In different experiments in which features of the nest box were modified or different group sizes were compared, the proportion of consistent nest box layers ranged from about 60-90% of hens.

In experiment 1, involving 112 Hy-Line Brown hens, the first 20 eggs laid per bird were collected to measure corticosterone concentrations (as a measure of stress and welfare) in egg albumen. There were no differences due to nest box or group size (2-, 4- or 8- birds per cage).

At 23 weeks of age the presence of a nest box resulted in 33% higher plasma corticosterone concentrations, suggesting birds in cages with a nest box were more stressed. In the experiment, where birds had a minimum of 600 cm^2, there were no effects of group size. At 30 and 37 weeks there were no effects of nest box, but at 37 weeks cages with 4 or 8 hens had higher plasma corticosterone concentrations than cages with 2 hens. This effect of group size is known from the literature.

At 23 weeks of age, in cages with a nest box, birds that were classed as either 100% floor layers or 100% nest box layers (based on the previous 10 eggs laid) showed elevated corticosterone concentrations in egg albumen. However, the relationship was relatively short-lived and subsequently was not found at 29 or 35 weeks of age.

Corresponding to the higher corticosterone concentrations at 23 weeks of age, the synchrony of egg laying was higher in cages with a nest box, suggesting social factors around this age may be increasing the level of stress in the presence of a nest box. This also coincides with the time hens are determining their preferred egg-laying site.

While the result may be associated with some form of social competition for a resource, presumably the nest box, it is contrary to the perception that hens will be stressed if they are unable to lay in a nest box. As mentioned already, the presence of a nest box in the cage increased the synchrony of egg-laying prior to 24 weeks of age. Competition amongst the hens for preferred egg-laying sites, in this case the single nest box in the cage, was probably greater as the timing of egg laying became more synchronised. This may help explain why birds in cages with a nest box were found to have 33% higher plasma corticosterone concentrations at 23 weeks. The extent of the assumed competition for the nest box may also be relevant to determining whether hens are excluded from using the nest box and become floor layers.

There was no evidence of immuno-suppression, based on heterophil to lymphocyte ratios.

Thus, none of the stress-related measurements were suggestive of any long-term change in HPA function and hence a chronic stress response.

Manipulation of the light-dark schedule to introduce a 3 h period of light during the night commencing at midnight, resulted in a shift in egg laying time with about 3 quarters of the hens laying a proportion of their eggs in darkness (especially between 03.00 and 06.00 h), but did not alter the synchrony of egg laying times.

There were no effects of consistently laying in the dark or light on any of the stress physiology parameters measured. When egg-laying coincided with darkness, hens did not perform the typically active pre-laying ‘searching’ phase of nesting behaviour. The manner of inserting a period of light during the night time, whether by a gradual introduction over weeks or an abrupt introduction, did not result in a stress response for hens.

As most eggs laid by hens in the dark are laid on the wire floor with no impact on stress levels, this suggests that the nest box may not be important to hen welfare, at least when egg laying occurs in the dark.

Increasing the number of birds per nest box from 2- to 8- did not affect the proportion of eggs laid in the nest box. While it was not possible for all 8 birds to simultaneously occupy the one nest box in a cage, in one cage up to 5 birds were regularly observed to co-occupy the nest box. In this cage all 8 birds generally laid in the nest box each day. However in other cages, including one cage with 2 birds, neither bird used the nest box for egg laying. Clearly there are social factors within the cage environment influencing hens’ preference for laying in the nest box.

1.2 Minor findings

This project showed a consistent choice by individual hens for laying eggs either in the nest box or on the wire floor; this consistency of site selection provided a model whereby for the first time, the impact of nest site manipulation on stress physiology (and behaviour) could be determined in other experiments.

While increasing the number of birds per nest box from 2- to 8- did not affect the proportion of eggs laid in the nest box, social factors associated with group housing (8 birds per cage) compared to single housing appeared to reduce the proportion of nest box eggs. Although the latter finding was not statistically significant, the difference due to birds per cage (1- vs. 8- birds) was prominent in one of the 2 experimental rooms. The ambient temperature in the affected room was slightly lower and this may have been a relevant factor in increasing the motivation of singly-housed birds to lay in nest boxes. Alternatively, a warmer ambient temperature may have decreased the motivation for birds to use nest boxes for egg laying.

2 The importance of nests for the welfare of laying hens in cages.

2.1 Background and literature

The majority of commercial laying hens in the world are housed in cage systems in contrast to non-cage systems such as aviaries, barns or free range (van Horne and Achterbosch 2008). In Australia about 80% of laying hens are housed in cages (Runge 2006). Housing laying hens in cages is a controversial animal welfare issue for the egg industry with impacts on public acceptance of current industry practices and the potential to lead to imposed changes which may be associated with increased capital costs. Indeed, due to concern for the welfare of laying hens in different production systems in the European Union (EU) over the last few decades, EU Directive 1999/74/EC sets down minimum standards for the protection of laying hens in legislation (EU 1999; Blokhuis and van Niekerk 2005). One outcome in the EU is that the use of conventional cages for laying hens will be banned from 2012 onwards. Cage egg production may continue, however with the use of modified cage designs such as ‘furnished’ cages. Furnished (or enriched) cages incorporate a perch, a nest box, a dust bath and claw trimmer (Appleby and Hughes 1995; Barnett et al. 2005). Coupled with fulfilling the EU Directive is a large EU project (LayWel) reviewing the knowledge on hen welfare in relation to their housing (LayWel 2006). While the Australian egg industry has been given some certainty regarding cages until about 2015, the hen welfare standards in the EU are likely to place ongoing pressure on the Australian industry for change.

2.2 Furnished cages

There are a number of recent European reports regarding commercial egg production in furnished cage systems in which hen mortality, egg production, egg quality, feed consumption and other parameters have been evaluated (Guesdon and Faure 2004; Croxall et al. 2005; Roll et al. 2005; Tauson and Holm 2005; Hulzebosch 2006). To quantify the benefits or otherwise of furnished cages under Australian conditions, an AECL- / DPI-co-funded research project (Barnett and Cronin, 2005) was conducted between 2002-2004 to evaluate the commercial Victorsson Trivselburen 8-bird Furnished Cage (Sweden). Specifically, the project investigated the effects of the different components of the cage furniture, viz. nest box, dust bath, perch and their interactions via a factorial-design experiment, to determine the effects of the cage furniture on bird welfare, behaviour and egg production (Barnett and Cronin 2005). The project found that with the exception of the perch improving bone strength, cage furniture provided no quantifiable welfare benefits compared to hens in cages without furniture, although the ‘furniture’ was well-used by the birds. The frequency of use of the perch and dust bath in the furnished cages were reported by Barnett and Cronin (2005) and Cronin et al. (2006); while these corresponded to data from on-farm surveys in Sweden by Tauson and Holm (2005) that survey generally found a higher proportion of eggs laid in the nest boxes than in the Australian studies. In relation to nest box use, although we found 62% of eggs were laid in nest boxes, about one-third of eggs from hens with access to a nest box were laid outside the nest box, on the wire cage floor (Barnett and Cronin 2005; Cronin et al. 2005).

2.3 Floor eggs in cages furnished with a nest box

While the majority of eggs in furnished cages are laid in the nest box, egg laying outside the nest box on the wire cage floor can be a common problem. For example, in some of the earlier work on cages modified to incorporate a nest box, Sherwin and Nicol (1992) reported that floor eggs ranged from 11-25%. The incidence however, decreased with increasing age of the birds. Similarly, Cooper and Appleby (1996) reported an overall 13% floor eggs, but found that floor eggs declined from 25% in the first week of lay to 5% after 6 weeks. In subsequent work investigating the effects of rearing background, age at placement in cages, time of day and social interactions by Sherwin and Nicol (1993a), there was an overall 14% floor eggs. More recently, Wall et al. (2002, 2004) investigated different nest box/cage designs and compared 2 strains of birds, and reported the incidences of floor eggs ranged between 10-90% and 10-33%, respectively, and Guesdon and Faure (2004) reported that the incidence of floor eggs ranged from 32-57% in a comparison of 4 designs of furnished cages. In our research on furnished cages (Barnett and Cronin 2005), we reported that while the overall incidence of floor eggs was 38% (across all cages with a nest box), for cages with the full complement of furniture items (nest box, dust bath and perch) the incidence of floor eggs was 21% of eggs laid. The reduction in floor eggs was probably due to the perch. The occurrence of floor eggs in cages with and without a perch was 45% compared to 31% (Cronin et al. 2005).

Nevertheless, the occurrence of floor eggs in the Australian experimental situation differs from the results of surveys by Tauson and Holm (2002, 2005) on Swedish poultry farms using furnished cages, who found very low incidences of floor eggs (0-10% of eggs).

In comparison, floor eggs may also be a problem in non-cage housing systems. The incidence of floor eggs in an aviary system was reported by Abrahamsson and Tauson (1995) to range from 0.7 and 18.4%, whereas in an experimental pen situation, Zupan et al. (2008) reported that 17 of 24 hens (71%) were consistent nest box layers whereas the other 7 birds (29%) preferred to lay in a litter tray without a cover.

2.4 Welfare and economic consequences of eggs laid outside the nest box

Dawkins (1988, 1990) and Cooper and Appleby (1996) have suggested that floor laying may be indicative of a welfare problem if hens have been thwarted from performing nesting behaviour. Notwithstanding the argued relationship between nesting behaviour / nest boxes and the welfare of laying hens (Duncan 2001; Weeks and Nicol 2006), there is a practical relevance of reducing floor eggs in cages containing a nest box. Guesdon and Faure (2004) for example, reported that a higher proportion of eggs laid outside the nest boxes in furnished cages were cracked and or/dirty thus devaluing them to the producer. A production, and thereforeeconomic consequence for egg farmers,might be that producers take longer to achieve the optimum cash flow due to the higher proportion of lower grade and broken eggs, and thus reduced return on the capital investment from installing cages with nest boxes. The situation that perhaps one-third of eggs are laid outside the nest box is an issue that requires consideration for the farmer’s costs and economic returns.

2.5 Are nest boxes important for the welfare of hens in cages?

Many questions are therefore raised in relation to nest boxes and hen welfare, such as: Why do some hens choose not to lay in a nest box? Are hens that lay in the nest box or on the wire floor consistent in their choice of egg-laying location and site within that location? What do hens perceive as a 'suitable' nest site? What factors influence this decision? If hens choose not to lay in a nest box is their welfare adversely affected? One major issue in the welfare debate for the egg industry concerns whether nest boxes are important to hen welfare. A key objective was to examine the importance of nests (ie. nest boxes) with respect to the welfare of laying hens in cages.

2.5.1 Pre-laying behaviour of hens

From studies of pre-laying behaviour of domestic hens, it is generally accepted there are 2 phases of pre-laying behaviour (Sherwin and Nicol 1992). Beginning one to 2 h prior to oviposition, the activity level of hens increases in a phase of behaviour termed 'searching' in which hens appear motivated to seek a nest site. In this phase hens increase locomotion and perform behaviours such as inspection of potential nests. An important function of pre-laying activity in wild populations would seem to facilitate selection of a secluded and secure place to incubate the clutch of eggs (McBride et al. 1969; Duncan et al. 1978). For example, Duncan et al. (1978) reported that nest sites were consistently inaccessible and concealed fromhuman observers. In domestic situations, 'dark' places such as provided by a nest box, are often chosen by hens for egg laying. Once hens have selected the preferred nest site, the 'sitting' phase commences. This phase includes the adoption of a sitting posture, interspersed with nest-building activities such as scratching the floor and/or litter rotating the body on the nest and collecting any available litter. Nevertheless, compared to other avian species, the nest-building activity of domestic laying hens is typically rudimentary (Duncan 1980). In wild situations, the broody hen remains sitting after laying the final egg in the clutch (McBride et al. 1969). From an evolutionary perspective, these pre-laying behaviours probably ensure the selected nest site is secluded enough to keep the sitting hen safe from predators.

Activities performed in the searching phase are goal-directed or appetitive behaviours, occurring when hens are motivated to find a suitable nest for oviposition (the consummatory behaviour). Thus, Appleby and McRae (1986) and Duncan and Kite (1989) showed that hens were motivated to lay their egg in a nest box, and if a nest box was not available hens performed more nest-searching behaviour (Cooper and Appleby 1995; Freire et al. 1996). While an increased occurrence of appetitive behaviour may indicate a stronger motivation to achieve the consummatory phase, it does not necessarily indicate that increased pre-laying locomotion reflects increased frustration and thus a potential welfare problem. For example, using an aversive task approach, Freire et al. (1997) suggested that hens were only weakly motivated to reach the nest site during the searching phase, although the motivation to gain access to a nest site increased near the start of the sitting phase. Cooper and Appleby (2003) found that hens trained to push open a door to reach a nest site worked hardest 20 min prior to oviposition, compared to 40, 60 or 80 min. Interestingly, the latter authors also measured the work rate of hens to leave the nest pen after egg laying and return to a home pen. The home pen contained food, water, litter and a perch. At the maximum applied time of 4 h post-oviposition in the nest pen, the work rate exerted by hens was equivalent to that 40 min before oviposition to enter the nest pen.

2.5.2 Pre-laying behaviour and oviposition

The control and expression of pre-laying and nesting behaviour and oviposition by hens have been reviewed by Duncan (1980) and Appleby et al. (2004). Although the egg that is ovulated today is not laid until about 25 h later, the egg spends about 20 of these h in the shell gland (Appleby et al. 2004). Nevertheless, today's ovulation has a close physiological relationship with the nesting behaviour performed for the egg laid today (oviposition). Appleby et al. (2004) describe oviposition thus: "Once oviposition begins, the sphincter between the shell gland and the vagina relaxes, the shell gland contracts, the hen increases abdominal pressure and the egg is laid by passing though the vagina, cloaca and vent. After the remnants of the follicle have ruptured and released the ovum, its remnants form the post-ovulatory follicle. This has an important role – it secretes oestrogen and progesterone, which control the onset of pre-laying and nesting behaviour 24 h later, just prior to the laying of the egg (Wood-Gush and Gilbert 1964, 1973). Selection for egg number, together with ad libitum access to food, has transformed Gallus gallus from the jungle fowl that, under natural conditions, lays a clutch of 10-20 eggs, through primitive varieties such as Indian village fowls typically laying 40-50 eggs in a year, to the modern laying hybrid. Its very highly developed oviduct, together with its liver where lipid for the yolk is synthesized, produces over 300 eggs in 365 days. Initially ovulation occurs every 24-25 h but, as oviducal senescence takes place, the interval lengthens and sequences of eggs, which are separated by a non-laying day, become shorter."

Pre-laying behaviour of birds and the timing of luteinizing hormone (LH) release, and thus ovulation and oviposition, however, are also influenced by light. Wilson and Cunningham (1984) have demonstrated that the increase in LH that initiates an ovulation in hens is restricted to an 8-10 h period in the middle of the night, which is termed the 'open-period'. Manipulation of the timing of dawn (lights on), but more importantly dusk (lights off), can alter the time of ovulation and oviposition (Morris 1973; Lewis et al. 2007a). Lewis et al. (2007a) also comment that by turning the lights on earlier whilst keeping lights-off unchanged, may prevent adjustment of the hens' ovulatory cycle and oviposition to occur before lights-on. The consequence of this is relevant to non-cage systems, as birds will lay in the dark outside the nest boxes. According to Lewis et al. (2007a), the only permanent way to minimise pre-dawn egg-laying in brown hybrid laying hens is to provide a photoperiod of at least 16 h.

Egg laying in the dark is an interesting phenomenon, since birds are mostly inactive in the dark (Tanaka and Hurnik 1991; Khalil et al. 2004). Sherwin and Nicol (1993a) for example, reported the incidence of nest box eggs decreased if hens laid during the dark period. If pre-laying behaviour is only relevant in hens experiencing light at the time, then the question needs to be asked: What is the function of pre-laying behaviour? For example, does pre-laying 'searching' behaviour function to move the bird to a darkened location for oviposition, and if the ambient environment is already dark, does the motivation to perform pre-laying activity decrease and terminate?

2.5.3 Pre-laying behaviour, nest boxes and welfare

There is no doubt that laying hens have a strong preference for access to discrete, enclosed nest-sites (Bubier 1996; Cooper and Appleby 1996). Further, studies have shown that hens are highly motivated in experimental situations to reach a familiar nest site, especially as oviposition approaches. For example, the motivation to access a secluded nest site for egg laying has been measured by how hard a bird will push through a small opening or its willingness to pass close to a dominant hen (Cooper and Appleby 1995, 1997, 2003; Freire et al. 1997, 1998).

In situ observations of pre-laying behaviour and oviposition by hens in cages with and without a nest box have been reported by many authors including Wood-Gush and Gilbert (1969), Appleby (1990), Sherwin and Nicol (1993b), Cooper and Appleby (1996), Cronin and Desnoyers (2005) and Shinmura et al. (2006a). Various levels of behavioural detail and activity of hens in the pre-laying period have been reported.

When a nest box was unavailable, hens were more active, engaged in locomotory behaviour for a longer duration before oviposition and often performed what has been described as stereotyped pacing (Duncan and Wood-Gush 1972; Wood-Gush 1972). Stereotyped pacing is a behavioural response that has been interpreted as a sign of frustration (Wood-Gush and Gilbert 1969; Zimmerman et al. 2000; Yue and Duncan 2003; Appleby et al. 2004). Wiepkema et al. (1983) defined stereotyped pacing as a form of restless locomotion, in which the bird steps higher than normal and typically, performs the action with a frantic and stereotyped character. Yue and Duncan (2003) compared the pacing behaviour of hens in cages with a nest box, without a nest box and when access to the nest box was blocked over 3 7-day periods when hens were 28, 32 and 36 weeks of age. Hens with access to the nest box spent significantly less time pacing in the hour before egg laying (7%) compared to hens that had no experience of a nest box (23%) or who had their nest box blocked (20%). It also found no difference in behaviour over time suggesting that hens did not adapt, at least behaviourally, to the lack of a nest box.

Abnormal behaviours such as stereotyped pacing in the absence of a suitable nest site are considered by some to be behavioural pathologies and thus indicative of a welfare problem (Appleby 1998; Duncan 2001; Keeling 2004). Further, ‘normal’ nesting behaviour is considered essential for laying hen welfare (LayWel 2006). Although Wood-Gush (1982) later concluded that oviposition in conventional cages without a nest box leads to the performance of abnormal behaviour, he also noted that the type of abnormal behaviour shown by laying hens varied between strains and appeared to be under genetic control. Wood-Gush thus considered the possibility to breed birds that were not disturbed by the conventional cage for laying (1982).

Zimmerman et al. (2000) used the occurrence of a specific vocalisation of laying hens, the ‘gakel call’, as a behavioural response to thwarting different behaviours in laying hens. The gakel call is reported to be indicative of frustration in hens (Zimmerman and Koene 1998; Keeling 2004), but is also referred to as the pre-laying call, which is typically given during the searching phase of pre-laying behaviour when hens are housed in floor pens with nest boxes (Wood-Gush and Gilbert 1969). Zimmerman et al. (2000) reported a significantly higher frequency of gakel calls when hens were ‘thwarted’ from nesting by removing them from their nest boxes during the sitting phase of pre-laying behaviour. Since Zimmerman et al. (2000) only tested the birds on a single occasion, it is difficult to conclude whether this vocalisation reflects frustration within the context of nesting or whether the frequency of calling would change after repeated experience of removal from the nest box.

Nevertheless, Dawkins (1990) argued that laying hens 'suffer' when deprived of suitable nest sites. Dawkins (1990) considered suffering to refer to a wide range of prolonged or acute, unpleasant subjective states (e.g. boredom, frustration, thirst). Such states appear to have evolved by natural selection as a means of avoiding danger or restoring physiological deficits resulting from an animal's natural environment. Subsequent authors including Sherwin and Nicol (1992), Duncan (1995, 2001) and Weeks and Nicol (2006) have restated Dawkin's opinion on the absence of a nest box and poor welfare, even though they state the use of nest boxes by hens can be quite variable. The finding that most hens lay in a nest box when provided is a major argument supporting the belief that a nest box is important to hen welfare (Weeks and Nicol 2006). Keeling (2004) refined this reasoning by suggesting that "if a hen is motivated to lay in a nest, but cannot find what to her is an appropriate site so, as a last resort, lays in an inappropriate place, then it probably is a welfare problem".

2.5.4 Pre-laying behaviour, nest boxes and welfare

Although behavioural evidence is cited to indicate nest boxes improve hen welfare, there is little physiological evidence supporting the behavioural evidence. While few studies have specifically examined the effects of nest boxes on physiology, Guesdon and Faure (2004) and Barnett et al. (2005) found no effects of the presence of 'furniture', including a nest box, and Beuving (1980) found no effects of a nest tray with litter, on adrenal responsiveness of laying hens.

2.6 Welfare and economic consequences of eggs laid outside the nest box

This project had 3 main objectives. These were:

1) To investigate the influence of 3 environmental factors (nest box, group size and light regime) on egg laying, the consistency of egg-laying site and hen welfare.
2) To examine the implications of manipulating light-dark schedules for hen welfare and the timing and synchrony of oviposition, including oviposition in the dark.
3) To investigate the influence of nest design features and social factors on nest box use.

3 Experiment 1 – the effects of a nest box, group size and light schedule on egg laying, consistency in choice of egg-laying site and hen welfare.

3.1 Background

Conventional cages have been criticised for reducing the welfare of laying hens. The welfare concerns derive from a combination of factors including the type of housing (cage compared to non-cage system) and the (limited) space and (lack of) facilities allotted per bird. Opponents of conventional cage housing for laying hens argue that such systems do not satisfy the 'freedom to express normal behaviour' condition described under the 5 Freedoms Concept (Farm Animal Welfare Council 1992). According to Duncan (2001), the absence of a nest box in cages was perhaps the most serious welfare issue for laying hens, while the LayWel (2006) project concluded that hens should be provided a discrete, enclosed nest site for egg laying. Other welfare issues also exist for laying hens, including the impact of husbandry procedures such as beak trimming, the consequences of poor health and hygiene standards, social problems including aggression and cannibalism and the development of vices such as feather and vent pecking, and the competency of the stock person to manage birds.

Nest boxes have been designed and manufactured for incorporation in modern cages. Nest boxes enable hens to perform their species-specific pre-laying and nesting behaviours, although nesting behaviour without substrate may be limited. Without a suitable nesting site (e.g. the nest box), and if hens are frustrated in the period leading up to oviposition, it should be possible to detect physiological responses in birds that are indicative of acute (short-term) and chronic (long-term) stress, and thus comment on the risks to hen welfare.

3.2 Objectives and hypothese

The major single objective of this experiment was to investigate the importance of nest boxes for the welfare of laying hens. To achieve this objective we applied 3 environmental factors known to influence egg laying behaviour.

The factors were:

1) presence of a nest box,
2) group size (as group size increases, without increasing the number of nest boxes, pre-laying behaviour may become disrupted, particularly if there is synchrony of oviposition), and
3) light schedule (light has important stimulatory and synchronising effects on the time of ovulation and egg laying in hens, as well as on the activity level of hens in general).

3.2.1 Hypotheses

- The presence of a nest box in the cage reduces the risks to bird welfare, based on stress physiology.
- Birds that are floor layers have poorer welfare than nest box layers, based on stress physiology.
- Increasing the ratio of birds per nest box reduces the proportion of nest-box eggs.
- Increasing the ratio of birds per nest box increases the risk to bird welfare based on stress physiology.
- Manipulating the light to dark schedule to introduce a period of light during the 'night' alters the timing and synchrony of egg laying.
- Birds that lay in the dark have poorer welfare than birds that lay in the light. Birds that lay in the dark do not perform pre-laying or nesting behaviour.

3.3 Materials and methods

112 Hy-Line Brown hens were introduced at about 16 weeks of age (replicate 1 at 17 wks; replicate 2 at 15.8 wks) to 12 modified, commercial Victorsson Trivselburen 8-bird furnished cages (Sweden) located in 2 adjacent climate-controlled rooms (6 cages per room). The perch and dust bath had been removed from all cages and only half of the cages had a nest box. Day length was increased each week by 30 min until the hens were exposed to 16 h light per day at 22 weeks of age. Day-time shed temperature ranged from 22-25°C while night-time temperature was about 18°C.

The experiment had a 2 x 3 x 2 factorial design. The main effects were:

- Nest box - present vs absent
- Group size - 2, 4 vs 8 birds/cage
- Light schedule - standard schedule of 16h light (L):8h dark (D) vs modified light schedule of 13hL:5hD:3hL:3hD; the latter commenced from ~170 days of age (24 weeks of age).

There were 2 replicates in time. Replicates commenced in May 2005 and March 2006, respectively. In each replicate, each treatment combination was represented in each room. Nest box and group size treatments were allocated to cages at random within rooms and replicates, and the light schedule treatments were allocated to the rooms at random. The lighting schedule in each room was controlled independently. The birds in the 2 replicates were from different hatcheries.

The 6 experimental cages per room were positioned in a back-to-back formation on the upper tier of a bank of cages containing 2 tiers of ten cages. There was a cage with hens on either side and at the rear of each treatment cage. Photograph 1 was taken before birds were placed in the cages and shows the bank of cages in one of the experimental rooms. The lower tier was used to house non-experimental (sentinel and spare) birds.

Cages measured 1.2 metres (m) wide, 0.5 m deep and 0.45 m high at the rear of the cage. The nest box (if present) was located at the right side of the cage and measured 0.24 m wide, 0.5 m deep and 0.27 m high at the front, and was covered by a vinyl flap. A piece of 'astro turf' (0.37 m x 0.22 m x 15 mm thick) overlaid the wire floor in the nest box. Feed and water were available ad libitum.

Photograph 1 - One bank of cages before placement of the birds.

3.3.1 Video recording of hen behaviour and oviposition

Continuous video records were made of all experimental birds from the day of introduction to the cages until 38 weeks of age. Video cameras with built-in infra-red (IR) lights were positioned above and below cages as well as inside nest boxes (if present); this provided visibility at all times. Spiral leg bands were placed on the hens on the day of introduction to the cages. The leg bands were either white or black and were applied so that each bird within a cage had a unique combination of leg bands. In addition, birds were marked on the head and back feathers with carbon-based ink, as described previously by Cronin and Desnoyers (2005). Carbon in the black plastic leg bands and the ink absorbs IR light. The IR light is therefore not reflected back to the camera and its absence is visualised on the video monitor as a black shape. The combination of markings applied to the birds thus enabled their individual identification under IR light on the video record (see photographs 2a and b).

Photograph 2a and b - Digital images captured from video records showing views from below a cage of 8 hens (left) and above a cage of 4 hens with a nest box (right). The black leg bands and carbon-based ink marks on the back and head feathers register as black marks due to absorption of the infra-red light emitted by the cameras, thus enabling identification of birds.

For the first 8 weeks of the experiment, the date, time and location of all eggs laid by the experimental hens, that is in the nest box or outside the nest box on the wire cage floor, were recorded from the continuous video record. Over the next 13 weeks, oviposition data were sampled on 3 days per week - Tuesday to Thursday. A sample of digital images captured from the video record are presented in photographs 3a, b and c.

Photograph 3a, b and c - Three views of oviposition: (3a) single bird in nest box (left), (3b) 2 birds in nest box with a third bird inspecting the nest box (centre) and (3c) an egg laid outside the nest box (indicated by the arrow, right).

3.3.2 Consistency of egg-laying site in cages with a nest box

The oviposition location for each egg laid by the 56 hens in cages with a nest box was first quantified according to where the egg was laid, that is whether it first contacted the nest box or wire cage floor, which is outside the nest box. Consistency of nest box use was thus defined according to the proportion of eggs laid in the nest box. To analyse the consistency of nest box use, the data were described in histogram form with cohorts of ten eggs per time period. For estimating consistency of egg-laying site by hens, including consideration of eggs laid outside the nest box (on the wire floor), the cage was arbitrarily divided into 5 zones representing one-fifth the width of the cage, equivalent to the area occupied by the nest box. The 5 zones were identified as far left (FL), mid-left (ML), centre (C), mid-right (MR) and nest box (NB). A numerical ‘score’ based on Pearson’s chi-square test was used to estimate consistency of egg-laying site. The Pearson's chi-square test is one of a number of chi-square tests used to assess 'goodness of fit' by reference to the chi-square distribution. The null hypothesis that the frequency distribution of certain events observed in a sample, viz. egg-laying sites, is consistent with a particular theoretical distribution is thus tested. The events considered must be mutually exclusive and have a total probability of one. The chi-square statistic was calculated as the number of eggs laid multiplied by the average (over birds) proportion of eggs laid in the particular zone. The Pearson's goodness of fit statistic provides a score of the consistency of laying in particular zones, allowing for the possibility that there may be different preferences. Higher scores indicate more consistency in egg-laying location, whereas lower scores indicate less consistency. Figure 3-1 provides a graphic of the range in scores that would be calculated for different numbers of eggs laid in cages, based on a 5-zone distribution. Higher scores result when proportionally more eggs are laid in fewer zones. For example, for a 5-zone distribution with 10 eggs, the scores can range from 0 to 40, respectively, for the circumstance in which 2 eggs are laid in each of the 5 zones [2, 2, 2, 2, 2] compared to all ten eggs in the same zone [10, 0, 0, 0, 0] (see Figure 3-1).

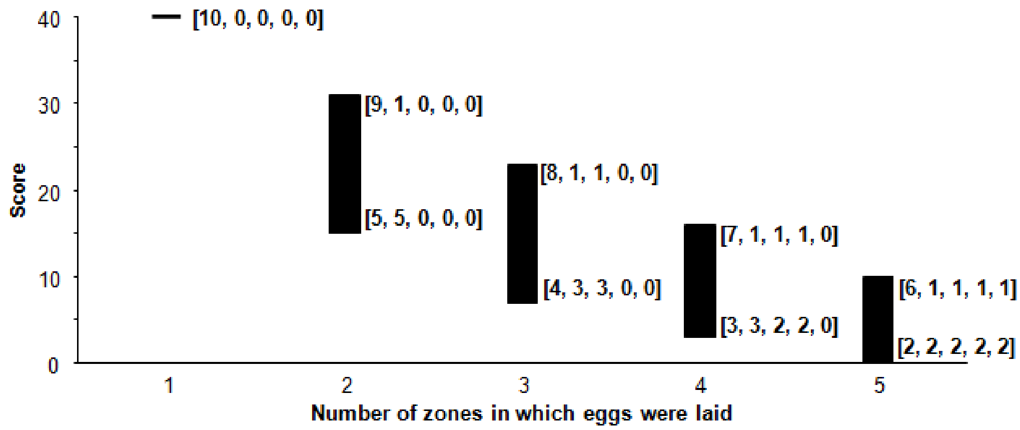


Figure 3-1 - The range in egg-laying site consistency scores, calculated using the Pearson's goodness of fit statistic. The figure shows the possible scores for 10 eggs laid by one hen, in different numbers of zones (from 1 up to 5) of a 5-zone distribution. The numbers at either end of columns show the number of eggs per zone to achieve the score.

3.3.3 Consistency of egg-laying site in cages without a nest box

For the 56 hens in the non-nest box cages, oviposition location was quantified via 2 methods. The first method was as for the nest box cages (see Section 3.3.2 above), which involved division of the cage into 5 zones, each representing one-fifth of the cage floor area, equivalent to the area of the nest box. As there was no nest box present in these cages, the space where the nest box would have been was termed far-right (FR). This method enabled statistical comparison of the consistency score measures between cages with and without a nest box. The second method for assessing the consistency of oviposition location involved dividing the cage into 15 zones on the wire cage floor using a 5 (cage width) x 3 (depth) grid. Each zone represented about 400 cm^2. This second method enabled the assessment of consistency of egg-laying site with more precision. For both methods, consistency of egg-laying site was estimated using Pearson's goodness of fit statistic for each bird, as described in Section 3.3.2 above. The scores ranged from a minimum of 5 (one egg per zone) to a maximum of 140 (ten eggs in one zone).

3.3.4 Welfare indicators – physiology and haematology measurements

The first 20 eggs laid by each hen were collected, and later identified from the video record, for measurement of egg albumen corticosterone concentrations. In addition, there were 3 sample collection periods during the experiment in which physiological and haematological samples were collected to assess stress responses of the hens.

3.3.4.1 Time lines for collection of the physiological samples

Week of age   Event (Procedure)

Data collection period 1:

23 Collection of eggs for egg albumen corticosterone assay (on one day)
23 Blood sample hens for plasma corticosterone assay (over 2 days)
24 Blood sample hens for haematology (on one day)

Data collection period 2:

29 Collection of eggs for egg albumen corticosterone assay (on one day)
30 Blood sample hens for plasma corticosterone assay (over 2 days)
31 Blood sample hens for haematology (on one day)

Data collection period 3:

35 Collection of eggs for egg albumen corticosterone assay (on one day)
36 Blood sample hens for haematology (on one day)
37 Blood sample hens for plasma corticosterone assay (over 2 days)
38 ACTH challenge and blood sample for plasma corticosterone assay (on one day)

The commencement of the modified light schedule, in which a 3 hour period of light was introduced from midnight, occurred after the haematology blood sampling in week 24 of age.

3.3.5 Sampling procedures and rationale

3.3.5.1 Collection of eggs for egg albumen corticosterone assay.

On days when eggs were to be sampled, the experimenter first made a grid map record to show the approximate location of all eggs in each cage, viz. in the nest box, on the roll-out tray or inside the cage. The grid map also identified the cage number, date, time of collection and initials of person recording the information and a sequence number was allocated to each egg marked on the grid. The sequence number was also written in pencil on the egg (shell). Eggs were then collected and placed on trays. Once all eggs in the room had been collected, the trays of eggs were taken to the laboratory where each egg was weighed whole before being broken to separate the albumen from the yolk. The albumen was weighed then frozen for later analysis of corticosterone concentrations. The video records were examined to match eggs to the hens that laid them. The frozen albumen was stored until transported to Camden, NSW where it remained frozen until analysed for corticosterone concentrations using the method developed by Downing and Bryden (2005). Total corticosterone concentrations in plasma were assayed using a commercial diagnostic kit (ICN ImmuChem Double antibody RIA, 7 Hills, NSW) under the supervision of Dr Jeff Downing, Faculty of Veterinary Science, University of Sydney.

Corticosterone transfers into albumen and accumulates during the period 3 – 5 h after ovulation (Downing and Bryden 2005) followed by a period of about 4 h of slow calcification when the shell membrane remains semi-permeable. The time between closing of exchange of corticosterone from the blood supply to the albumen occurs approximately 12 h (± 6 h) prior to egg laying, although the time period is not known with any certainty. Therefore, in the context of this experiment we do not necessarily anticipate egg albumen corticosterone concentrations will be indicative of events affecting the hen in the period she performs pre-laying behaviour (e.g. 2 hour pre-oviposition). Assuming oviposition occurs in the morning, the egg albumen corticosterone concentrations should reflect the blood plasma concentrations during the period of late afternoon and evening (night) when the hen will have different motivations - perhaps resting, roosting and sleeping.

3.3.5.2 Blood sample hens for plasma corticosterone concentrations.

As the majority of egg laying occurs during the morning, and egg laying per se affects corticosterone release (Beuving and Vonder 1977; Johnson and van Tienhoven 1981), blood sampling occurred between 13.00 and 14.00 h to avoid possible confounding effects of egg laying on stress measurements. In addition, to reduce the effects of the human on the birds, sampling occurred over consecutive days. For 2-bird cages, the 2 birds per cage were sampled on the same day with half the cages sampled each day. For 4-bird cages, 2 birds were sampled one day and the other 2 birds the next day. For 8-bird cages, 2 birds were sampled early in the schedule on the first day and a minimum of 45 minutes elapsed before another 2 birds were sampled. On the second day the other 4 birds were sampled, again with a minimum 45 minute period between the pairs of birds. The blood sampling sequence for the 2 rooms and cages within rooms was selected to minimise walking in front of cages that contained birds that were yet to be sampled on the day. For each bird, the time from capture to blood sampling was recorded as it is known that continuous immobilization, as occurs when the bird is held for blood sampling, results in corticosterone release (Beuving 1980). In other studies in which plasma corticosterone responses were measured for laying hens (e.g. Fraisse and Cockrem 2006), only blood samples collected within 3 minutes of capture were used. Similarly, in the present experiment, plasma corticosterone measurements from blood samples that took longer than 3 minutes to collect were omitted from the statistical analyses. A 3mL blood sample was collected from each hen by venipuncture from the wing vein using a 4.5 mL Monovette closed blood collection system (Sarsted Australia, Technology Park, SA), with heparin coating and with separation beads, and stored on ice. Blood samples were centrifuged at the completion of the session and the plasma frozen for later analysis of corticosterone concentrations at DPI At2od under the supervision of Dr Ian McCauley.

3.3.5.3 Blood sample hens for haematology and immunology.

Hens were blood sampled from the wing vein (not the wing used for the plasma corticosterone sample) for haematology and immunology parameters. Blood samples were assayed for a range of haematological parameters. The most pertinent variable for assessing welfare of birds is the ratio between heterophils and lymphocytes (H:L ratio). In response to stress this ratio increases. Approximately 2.5 mL of blood was collected into a 2.7 mL Monovette closed blood collection system (Sarsted Australia, Technology Park, SA), with EDTA coating and without separation beads. The whole blood remained in the Monovette on ice and was transferred to another laboratory for white blood cell counts (haematology) and immunology assays, within 1.5 h of the final sample being collected. The samples were assayed at CSIRO, Animal Health Laboratories, Geelong, Victoria.

3.3.5.4 ACTH challenge and blood sample for plasma corticosterone concentrations.

At 38 weeks of age, the response by all hens to an ACTH challenge was conducted to detect any changes in function of the HPA axis, particularly the presence of a chronic stress response. The procedure used was to inject ACTH (Synacthen; 12.5 IU/hen) as a single dose (0.5 mL), intra-muscularly, and measure corticosterone response 60 min later. While not unequivocal, the literature suggests that in response to an ACTH injection challenge, those animals that show higher cortisol/corticosterone concentrations are experiencing a chronic stress response and are thus less able to cope with their situation.

3.3.6 Statistical analysis

Analysis of variance was used to examine differences due to the main effects (GenStat 9.1, Lawes Agricultural Trust) on stress physiology parameters, egg-laying site preference indices and relevant bird characteristics. The experimental unit was the cage of birds and analyses were blocked on replicate and room. Differences due to replicate (batch of birds) and room (blocked on replicate) on bird age at the first, 11th and 21st egg were also examined by analysis of variance. Similarly, replicate effects were compared for differences in bird live weight, with bird age on the day of weighing used as a co-variate. Analysis of the haematological parameters occurred following log10 transformation. REML analysis (GenStat 9.1, Lawes Agricultural Trust) was used to examine the relationships between egg-laying characteristics of individual birds in cages either with or without a nest box, including the consistency of egg-laying site, nest box use and time of laying (light vs dark), and the different welfare parameters measured at the different sampling periods. Differences in the proportion of hens that laid single or multiple eggs per calendar day were analysed using the Chi-squared test.

3.4 Results

All 112 hens in experiment 1 were recorded to lay during the experiment. The mean age (± std dev) at first egg laid was 131.9 (± 8.22), while the minimum and maximum ages at first egg laid were 112 and 154 days, respectively.

3.4.1 Bird growth and development

3.4.1.1 Differences between replicates (batches).

Although birds of the same strain (Hy-Line Brown) were used for both replicates, biosecurity constraints required that the birds in the second replicate came from a different hatchery. Hens in the first replicate had lower mean body weight at the start of the experiment (first weighing occasion) than hens in the second replicate (Table 3-1). However, at the 3 subsequent weighing occasions there was no difference in live weight due to replicate and there were no differences due to the group size, nest box or light schedule main effects on live weight of hens on any of the 4 weighing occasions.

Table 3-1 - Effects of replicate (batch) on live weight of birds in Experiment 1, adjusted for age when weighed. Values shown are cage means.

Hen live weight (kg)	Replicate 1	Replicate 2	SED	P Value
at 115 days	1.522	1.668	0.0466	0.009
at 173 days	1.996	2.114	0.0666	0.103
at 216 days	2.202	2.255	0.0738	0.487
at 265 days	2.261	2.265	0.0785	0.962

SED: standard error of difference between the means.

3.4.2 Egg laying characteristics

3.4.2.1 Effects of replicate and room on the onset and progress of egg laying.

A possible consequence of the lower live weight for replicate 1 hens at the start of the experiment was a 10.4 day delay in the onset of lay (Table 2-2). There were significant (P<0.001) carry-over effects due to replicate of this initial delay on the age when hens laid the 11th and 21st eggs. However, although the means were significantly different between the 2 replicates, the differences due to replicate were reduced at both the 11th (7.8 days) and 21st eggs laid (5.6 days).

Tables 3-2 and 3-3. Effects of replicate and room on mean age at the 1st, 11th and 21st eggs.

Table 3-2 - Replicate effects

Hen age (days) at	Replicate 1	Replicate 2	SED	P Value
first egg	136.9	126.5	1.63	<0.001
11th egg	149.2	141.4	1.51	<0.001
21st egg	160.2	154.6	1.18	<0.001

SED: standard error of difference between the means.

There were no effects of room on age at onset of lay or age of the birds at the 11th or 21st eggs (Table 3-2). While every effort was taken to ensure the environmental conditions were similar in the 2 rooms, Room 1 tended to remain about 1ºC cooler than Room 2. This small temperature difference may have been due to the proximity of adjacent buildings, such that Room 1 was somewhat less protected from wind than Room 2.

Table 3-3 - Room effects

Hen age (days) at	Room 1	Room 2	SED	P Value
first egg	131.2	132.2	1.41	0.49
11th egg	145.2	145.4	1.58	0.93
21st egg	158.0	156.8	1.71	0.48

SED: standard error of difference between the means.

Effects of presence of a nest box and group size on onset and progress of egg laying: There was no difference in mean age at onset of lay between birds in cages with and without a nest box (Table 3-4). Thereafter however, the presence of a nest box in the cage delayed egg laying; birds in cages with a nest box reached their 11th (P=0.054) and 21st eggs (P=0.005) later than birds in cages without a nest box (Table 2-4). Group size had no effect on onset of lay or age of birds at the 11th and 21st eggs (P>0.05; Table 3-5).

Effects of a nest box and group size on mean age at the 1st, 11th and 21st eggs:

Table 3-4 - Nest box main effect (presence or absence of nest box in cage)

Nest box treatment:	Nest box	No Nest box	SED	P Value
Age at first egg (days)	132.9	130.4	1.63	0.154
Age at 11th egg (days)	146.9	143.7	2.14	0.054
Age at 21st egg (days)	159.4	155.4	1.18	0.005

Table 3-5 - Group size main effect (birds per cage; in 8-bird cages)

Group size treatment:	2 birds	4 birds	8 birds	SED	P Value
Age at first egg (days)	131.6	131.1	132.3	1.99	0.831
Age at 11th egg (days)	143.9	145.2	146.8	1.85	0.317
Age at 21st egg (days)	157.6	155.9	158.6	1.44	0.220

SED: standard error of difference between the means.
Values shown are cage means. There were no interactions.

3.4.3 Egg laying patterns – Consistency of egg laying site

3.4.3.1 Proportion of nest box eggs in cages containing a nest box:

As indicated in Figure 3-2, 44.6% of first eggs were laid in the nest box. While the proportion of nest box eggs increased from the first and the 7th eggs, from egg 8 to 40 the proportion of nest box eggs was relatively static, fluctuating around a mean of 70.4% (std dev 2.32). The values presented in the figure are the means pooled across treatments.

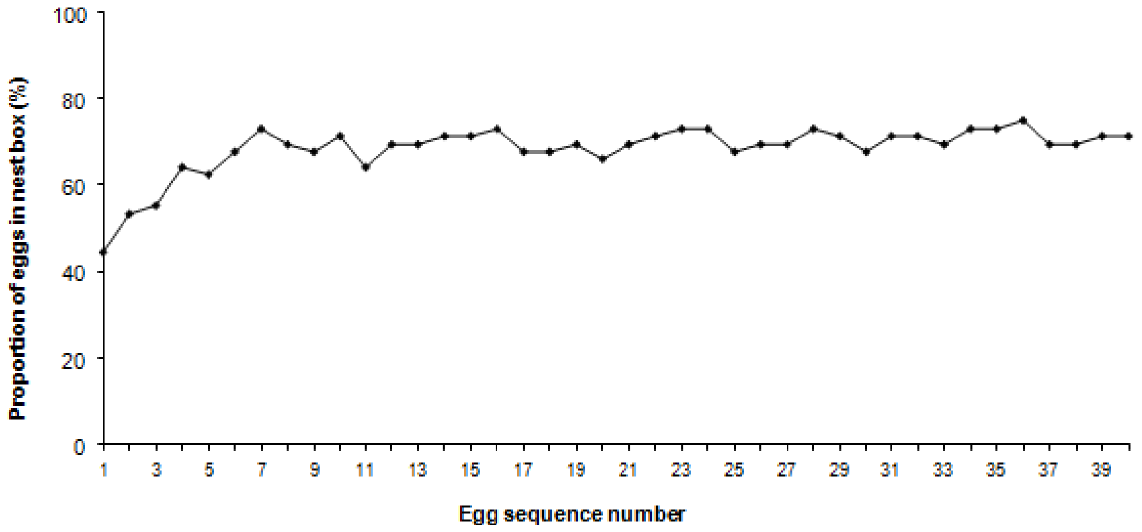


Figure 3-2 - The proportion of eggs laid in the nest box over the first 40 recorded eggs per hen; N=56. The values shown are the raw means pooled for all birds

Group size effects:

Figure 3-2 shows the change in the proportion of nest box eggs over the first 40 recorded eggs, based on the number of birds per cage. Although the 2-bird cages had the lowest proportion of nest box eggs (55%±10.9) from the 8h to the 40th eggs compared to 4-bird (74%±5.7) and 8-bird (69%±7.0) cages, it should be recognised the data are for relatively few (8) birds.

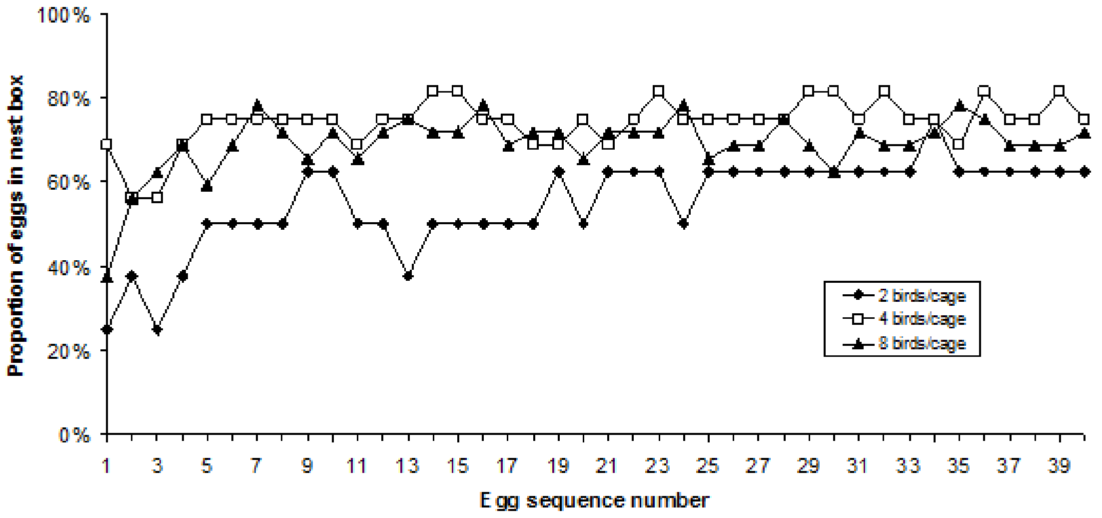


Figure 3-3 - The effects of group size on the proportion of eggs laid in nest boxes over the first 40 recorded eggs from cages containing 2- (n=8), 4- (n=16) and 8-birds (n=32). The data are raw mean values.

Replicate (batch) effects:

The effects of replicate, and thus batch of birds, on the proportion of nest box eggs over the first 40 eggs are shown in Figure 3-4. The raw mean (±std dev) proportions of nest box eggs from the 8h to the 40th eggs for birds in the 2 replicates were (62%±4.7) and (79%±4.3), respectively.

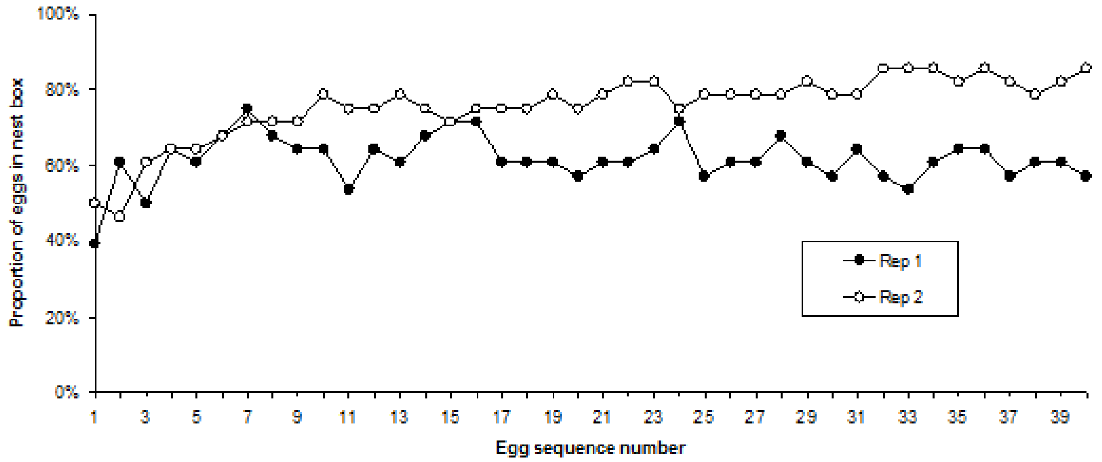


Figure 3-4 - The proportion of eggs laid in nest boxes over the first 40 recorded eggs for hens in the 2 replicates of the experiment. Although the birds in both replicates were Hy-Line Browns, the batches of birds were sourced from different breeders. The data are raw means for 28 birds per replicate.

Room effects:

Figure 3-5 shows the change in the proportion of nest box eggs according to room. From the 8h to the 40th eggs laid by the hens in Room 1 and 2, respectively, the proportions of nest box eggs were 76% (±3.0) and 61% (±3.5).

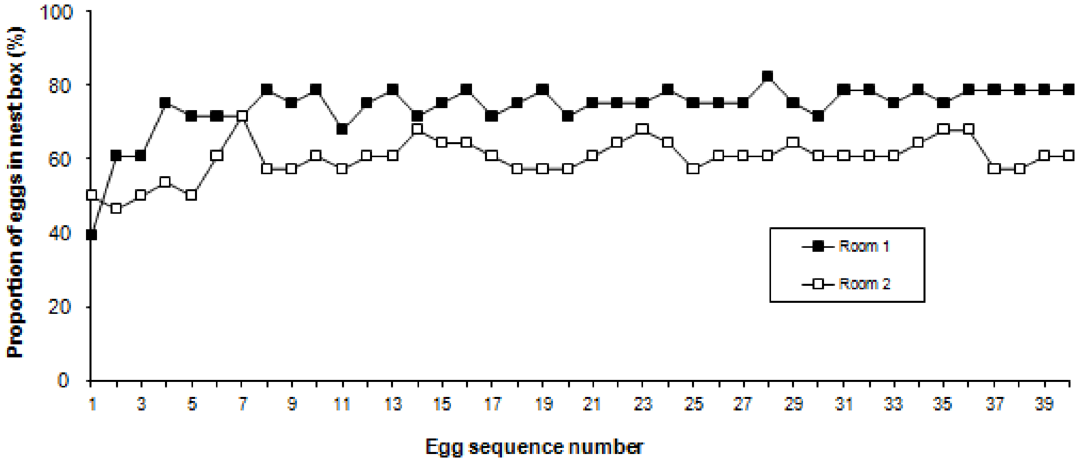


Figure 3-5 - The proportion of eggs laid in nest boxes over the first 40 recorded eggs for hens in the 2 experimental rooms. The data are raw means for 28 birds per room.

3.4.3.2 Consistency of egg-laying in the nest box:

The consistency of egg-laying in nest boxes by the 56 hens in cages with a nest box is first described by using histograms (Figure 3-6). The figure shows the number of hens that laid from 0-10 eggs per 10-egg cohort in the nest box. For the first 10 eggs laid, there was a broad distribution of eggs laid per hen in the nest box. From the eleventh egg onwards there is evidence of consistency of nest box use. For example, if consistency of laying was defined as ‘100% eggs laid in the same site’, then the number of hens that laid all eggs in the nest box was 15, 28, 33 and 31 hens, respectively, for the 10-egg quartiles. In comparison there were 8, 10, 12 and 11 hens, respectively, that laid zero eggs in the nest box in the quartiles. If the definition was less strict, for example 80% or more eggs in the same site, then 31, 37, 38 and 37 hens, respectively, laid 8 to 10 eggs in the nest box across the quartiles. The number of hens that laid 0 to 2 eggs in the nest box was 15, 16, 14 and 14, respectively. Of the remaining hens (inconsistent nest box layers that laid 3 to 7 eggs in the nest box), the number of hens per quartile of eggs was 10, 3, 4 and 0, respectively.

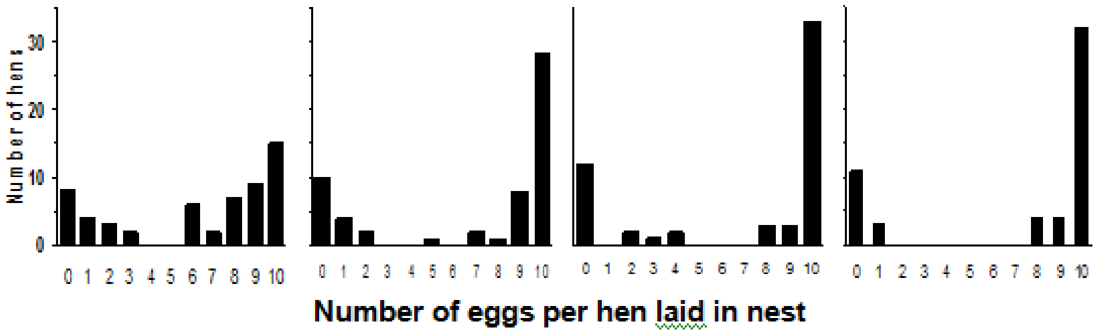


Figure 3-6 - Histograms of the number of hens laying in the nest box, from zero to 10 eggs, for the first (far left histogram) to 4th (far right histogram) quartile of 10 eggs laid; N=56 hens.

Consistency of egg-laying site (5-zone distribution) in cages with a nest box was also described using Pearson's goodness of fit statistic, calculated for each of the 56 hens over their first 40 recorded eggs (10 eggs per hen per quartile). The mean scores across the quartiles were 22.8, 29.1, 30.2 and 28.8, respectively. For comparison, the chi-square values for v=4 are 11.07 (P=0.05) and 18.47 (P=0.001). The score was lower than the P=0.05 significance level for only 16 of the 56 hens in the first quartile of eggs, then 10 of the 56 hens thereafter. The proportion of hens with scores greater than the P=0.001 significance level was 57% for the first quartile, and about 70% of hens thereafter.

Effects of group size:

Based on the proportion of nest box eggs over the 10 recorded eggs prior to the data sampling periods around 23, 30 and 37 weeks of age, there were no differences due to the group size main effect on the consistency of birds (in cages with a nest box) laying in the nest box (Table 3-6).

Consistency of laying in the nest box based on the proportion of nest box eggs per hen over 10 eggs.

Table 3-6- Consistency of egg laying in the nest box at 3 ages, for birds in groups of 2, 4 and 8 birds per cage. Values shown are the angular transformed percentage means. The back-transformed means are presented in parentheses.

Group size treatments:	2 birds	4 birds	8 birds	SED	P Value
Prior to 23 wks of age	43 (47%)	65 (82%)	62 (78%)	17.4	0.46
Prior to 30 wks of age	50 (58%)	73 (91%)	61 (76%)	21.1	0.59
Prior to 37 wks of age	68 (86%)	73 (91%)	67 (85%)	27.5	0.98

SED: standard error of difference between the means

Effects of light schedule:

From 24 weeks of age the light schedule in one of the 2 experimental rooms per replicate was modified through the introduction of a 3 hour period of light during the night, commencing at midnight. The birds in both treatments (rooms) nevertheless received the same total amount of light per 24 hour period. As shown in Table 3-7, there was a tendency (P=0.08) for birds in the Modified light treatment prior to 30 weeks of age to lay proportionally fewer eggs in the nest box than birds in the Standard light treatment. By the third data collection period (37 weeks) there was no difference between the treatments.

Table 3-7 - Consistency of egg laying in the nest box at 2 ages, for birds exposed to standard and modified light: dark schedules. Values shown are the angular transformed percentage means. The back-transformed means are presented in parentheses

Light schedule treatments:	Standard	Modified	SED	P Value
Prior to 30 wks of age	69 (88%)	52 (63%)	2.1	0.080
Prior to 37 wks of age	75 (93%)	63 (79%)	10.5	0.46

SED: standard error of difference between the means
Note – the Modified light schedule was imposed from 24 weeks of age; Light (L); Dark (D)
Standard light schedule: 16h L – 8h D; Modified light schedule: 13h L – 5h D – 3h L – 3h D

3.4.3.3 Floor laying sites in cages without a nest box, based on a 15-zone distribution:

Proportion of eggs laid in different zones within the cage:

The proportion of eggs laid in each of the 15 different zones in cages without nest boxes is shown in Figure 3-7. The figure is based on a total of 4,188 eggs, with an average of 74.8 eggs (±8.09) recorded per hen. The data suggest that, on average, all parts of the cage are used as egg laying sites.

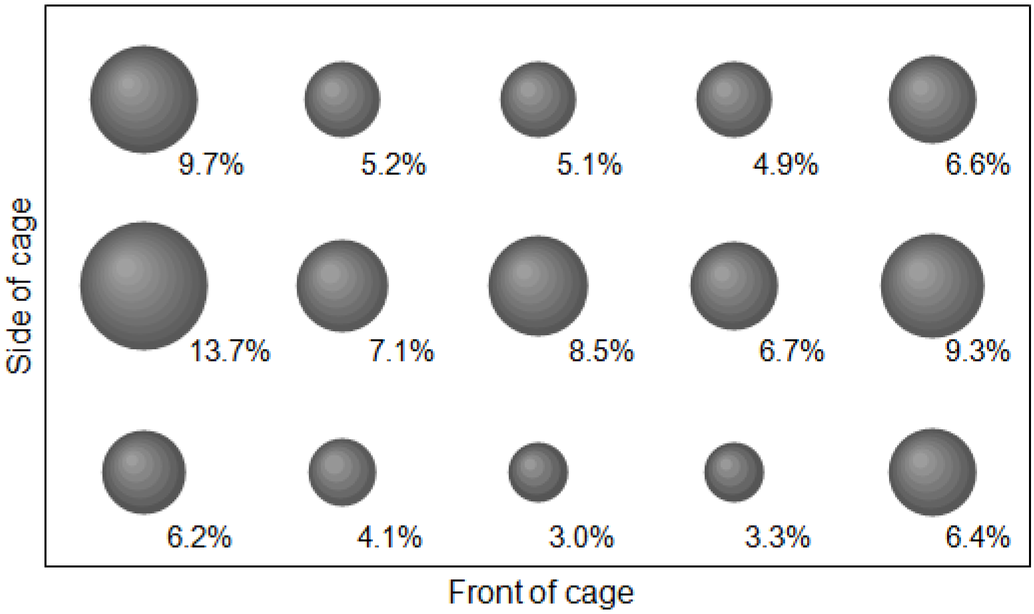


Figure 3-7 - Bubble graph showing the proportion of eggs laid in the 15 different zones in cages without nest boxes. The values are raw means, based on 4,188 eggs recorded for the 56 hens in cages without a nest box. Circle size indicates the relative proportion of eggs laid at that site. Each rectangular zone measured ~400cm^2.

To estimate consistency of egg-laying site on the wire mesh floor by individual hens, Pearson's goodness of fit statistic scores were calculated for each of the 56 hens. The mean (± std dev) Pearson's score for the 56 hens was 169.4 (± 131.78), while the median score was 136.1. Pearson's scores ranged from 14.7 to 671.9. Examples of birds with the lowest and highest individual Pearson's scores are presented in Figures 3-8 and 3-9, respectively. For comparison, the chi-square values for v=14 are 23.68 (P=0.05) and 29.14 (P=0.001), respectively. Only 2 of the 56 hens had scores lower than the P=0.05 significance level, indicating that their choice of egg-laying sites could have been at random. In comparison, based on the significance of the Pearson's scores, the majority (96.4%) of hens in cages without a nest box consistently laid in relatively few of the 15 possible egg-laying zones within the cages. As depicted in Figure 3-9, where a hen laid a high proportion of eggs in more than one zone, the zones were typically adjacent. Different orientation by the hen at oviposition could influence into which of the adjacent zones the egg was deposited.

On a 5-zone distribution basis, 51.9% of eggs were laid at either side of the cage, in the space equivalent to the nest box, suggesting a preference by at least half the birds for an oviposition site adjacent to a solid wall.

Bubble graphs showing the distribution of egg-laying sites for 4 individual birds:

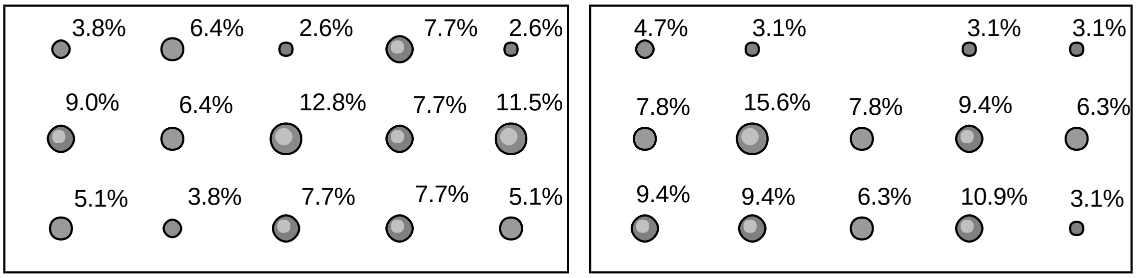


Figure 3-8 - Bubble graphs representing the proportion of eggs laid by 2 hens in different locations in cages without a nest box (15 zones). The hens had the 2 lowest consistency of egg-laying site scores based on Pearson's goodness of fit statistic. Left, Hen R131 from a 2-bird cage, score = 14.7, based on 78 eggs. Right, Hen R156 from an 8-bird cage, score = 21.3, based on 64 eggs.

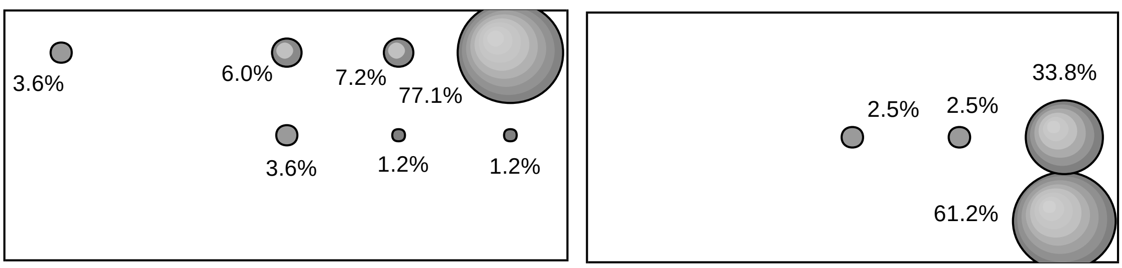


Figure 3-9 - Bubble graphs representing the proportion of eggs laid by 2 hens in different locations in cages without a nest box (15 zones). The hens had the 2 highest consistency of egg-laying site scores based on Pearson's goodness of fit statistic. Left, Hen R205 from a 4-bird cage, score = 671.9, based on 83 eggs. Right, Hen R229 from a 2-bird cage, score = 508.4, based on 80 eggs.

The change in the raw mean Pearson's goodness of fit statistic over the first 40 eggs laid is shown in Figure 3-10. The figure suggests that the level of consistency increases, estimated by higher scores, as egg number increases. For comparison, Figure 3-10 also shows the mean consistency scores for cohorts of 10 eggs laid by the hens prior to the 3 data sampling periods at 23, 30 and 37 weeks of age, adjusted for replicate, room and cage effects.

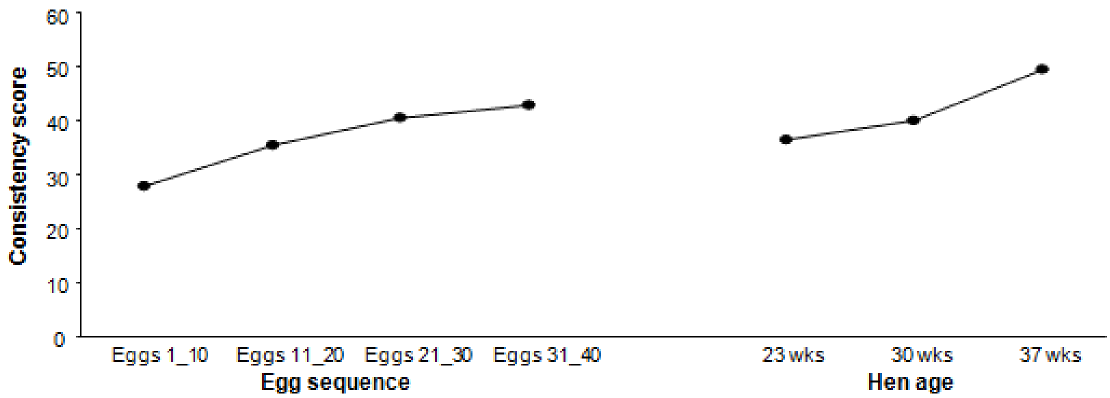


Figure 3-10 - Change over time in the consistency of egg-laying site, estimated using Pearson's goodness of fit statistic and based on a 15-zone distribution.

Effects of group size:

Group size did not affect the Pearson's goodness of fit 15-zone scores for consistency of egg-laying site by birds in cages without a nest box. Figure 3-11 shows the change in raw mean Pearson's scores over time for birds from cages with 2-, 4- and 8-birds, while Figure 3-11 and Table 3-8 show the cage mean values for birds in cages of 2, 4 and 8 birds over the course of the experiment as well as for the 10 eggs laid prior to each data sampling period, adjusted for replicate, room and cage effects.

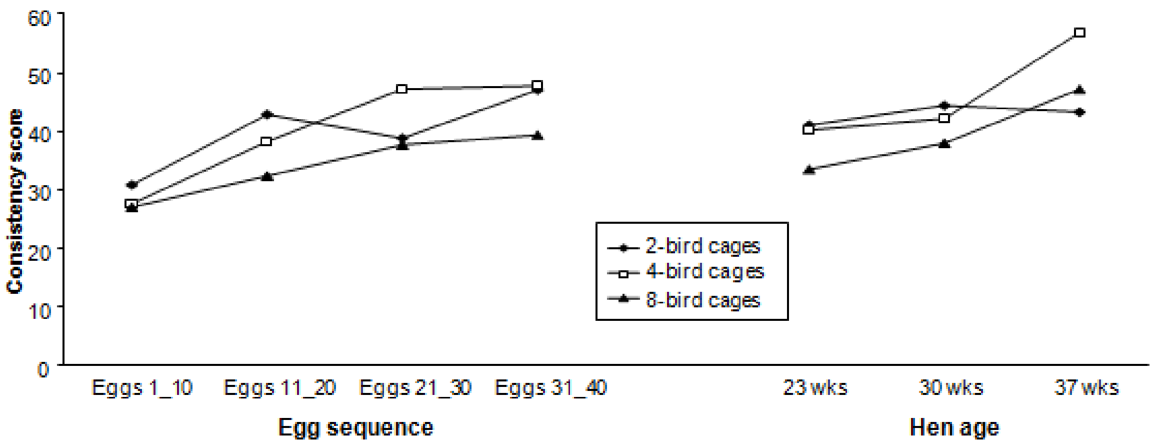


Figure 3-11 - Change over time in the consistency of egg-laying site, estimated using Pearson's goodness of fit statistic and based on a 15-zone distribution, for cages of 2-, 4- and 8-birds, over the first 40 eggs laid and in the ten eggs prior to 23, 30 and 37 weeks of age.

Table 3-8 - The effects of group size on consistency of egg-laying site estimated by the Pearson's goodness of fit statistic and based on a 15-zone distribution. Values are cage means.

Group size treatments:	2 birds	4 birds	8 birds	SED	P Value
Pearson's score for all eggs	186	175	162	56.3	0.92
Eggs recorded per hen (mean)	79.1	74.9	73.6	2.59	0.17
The 10 eggs recorded prior to:					
23 wks of age	41.0	40.2	33.5	13.18	0.83
30 wks of age	44.4	42.1	37.9	7.77	0.71
37 wks of age	43.2	56.8	47.1	10.92	0.49

SED: standard error of difference between the means

Effects of light schedule:

Light schedule did not affect the Pearson's goodness of fit scores for consistency of egg-laying site by birds in cages without a nest box. Table 3-9 shows the cage mean values for birds in cages in rooms exposed to Standard and Modified light schedules over the course of the experiment as well as for the 10 eggs laid period to each data sampling period.

Table 3-9 - The effects of light schedule on consistency of egg laying site, estimated by the Pearson's goodness of fit statistic. The Pearson's score for all eggs is based on individual bird data with the mean number of eggs recorded for which the scores were determined. The values for the 10 eggs recorded prior to the data sampling periods are based on cage averages.

Light schedule treatments:	Standard	Modified	SED	P Value
Pearson's score for all eggs	186	163	4.7	0.13
Eggs recorded per hen (mean)	76.4	75.4	1.54	0.61
The 10 eggs recorded prior to:				
23 wks of age †	40.7	35.8	4.10	0.45
29 wks of age	49.8	33.2	3.81	0.14
35 wks of age	54.0	44.1	12.31	0.57

SED: standard error of difference between the means
Note – the modified light schedule was imposed from 24 weeks of age; Light (L); Dark (D)
Standard light schedule: 16h L – 8h D; Modified light schedule: 13h L – 5h D – 3h L – 3h D
† Note, at 23 weeks of age the Modified light schedule treatment had not been imposed.

Figures 3-12, 3-13 and 3-14 show the proportion of eggs laid in different locations within cages without a nest box (based on a 15-zone distribution) during the whole experimental period, for cages with 2, 4 and 8 birds.

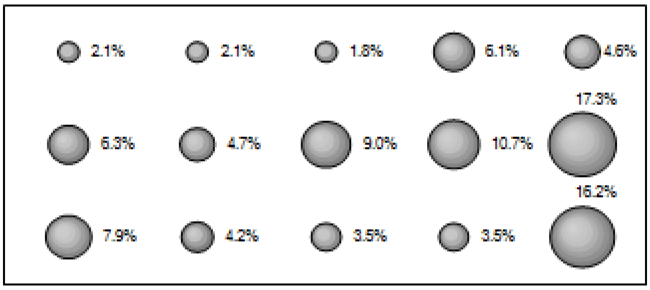


Figure 3-12- Bubble graphs showing the proportion of eggs laid in different zones in cages without nest boxes (based on a 15-zone distribution).

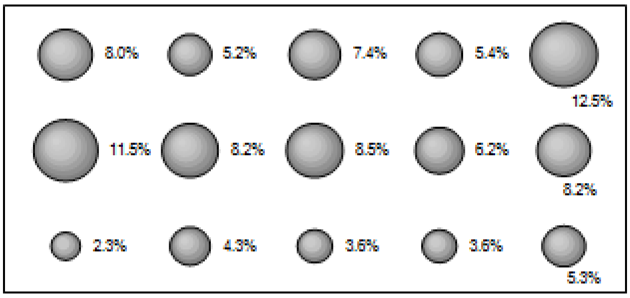


Figure 3-13 - Egg laying site distribution for cages with 2 birds. The figure is based on 633 eggs laid by 8 birds (ave. 79.1±5.46 eggs per bird).

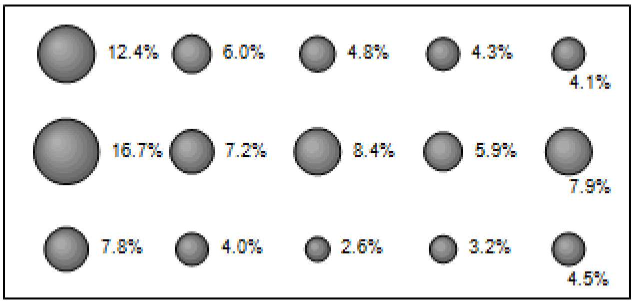


Figure 3-14 - Egg laying site distribution for cages with 4 birds. The figure is based on 1,199 eggs laid by 16 birds (ave. 74.9±5.25 eggs per bird).

3.4.3.4 Comparison of consistency of oviposition site between all cages (with and without a nest box), based on a 5 zone distribution:

To enable comparison of egg-laying site consistency between cages with and without a nest box, Pearson's goodness of fit scores were calculated for all 112 hens, based on the arbitrary division of cages into 5 zones equivalent to the space occupied by a nest box in the nest box treatment. While the presence of a nest box in the cage significantly increased the Pearson's goodness of fit consistency score during all periods, including during the initial 30 eggs laid (Table 3-10), there were no differences in consistency of oviposition site consistency score due to either the group size or light schedule main effects, and there were no interactions (Tables 3-11 and 3-12).

Consistency of egg laying site (5-zone) using Pearson's goodness of fit statistic (expected score of 4 if there were no individual preferences), based on cage averages:

Table 3-10 - Effects of the nest box on consistency of egg laying site (5-zone Pearson's score).

Nest box treatment:	Nest box	No Nest box	SED	P Value
Eggs 1 to 10	22	12	3.7	0.013
Eggs 11 to 20	28	14	2.9	0.00021
Eggs 21 to 30	31	15	2.4	0.000011
The 10 eggs recorded prior to:				
23 wks of age	27	15	2.9	0.0011
29 wks of age	31	17	3.4	0.0021
35 wks of age	32	19	3.2	0.0023

Table 3-11 - Effects of group size on the consistency of egg laying site (5-zone Pearson's score).

Group size treatment:	2 birds	4 birds	8 birds	SED	P Value
Eggs 1 to 10	15	20	17	4.5	0.61
Eggs 11 to 20	20	22	22	3.6	0.86
Eggs 21 to 30	24	23	23	3.0	0.97
The 10 eggs recorded prior to:					
23 wks of age	20	20	22	3.5	0.78
29 wks of age	22	26	24	4.1	0.70
35 wks of age	23	27	27	4.0	0.40

Table 3-12 - Effects of light schedule on the consistency of egg laying site (5-zone Pearson's score).

Light schedule treatment:	Standard	Modified	SED	P Value
The 10 eggs recorded prior to:				
29 wks of age	27	21	3.3	0.31
35 wks of age	29	23	2.7	0.28

SED: standard error of difference between the means
Note – the modified light schedule was imposed from 24 weeks of age; Light (L); Dark (D).
Modified light schedule: 13h L – 5h D – 3h L – 3h D
Standard light schedule: 16h L – 8h D

3.4.3.5 Consistency of egg laying in the light (compared to during darkness)

Consistency of egg laying in the light was calculated based on the proportion of eggs that were laid in the light in a sequence of 10 recorded eggs.

Effects of the presence and absence of the nest box on egg laying in the light:

The presence of a nest box in the cage did not affect the consistency of hens laying during the light period at any of the data collection periods (Table 3-13).

Consistency of egg laying in the light (based on the proportion of eggs per hen laid in the light over 10 eggs) at 3 ages, for birds with and without a nest box:

Table 3-13 - The effects of presence versus absence of a nest box in the cage on consistency of egg laying in the light. Values shown are the angular transformed percentage means with back-transformed means in parentheses.

Nest box treatment:	Nest box	No Nest box	SED	P Value
The 10 eggs recorded prior to:				
23 wks of age	77 (95%)	78 (95%)	5.6	0.82
29 wks of age	68 (86%)	74 (92%)	4.7	0.30
35 wks of age	78 (95%)	76 (94%)	4.1	0.68

SED: standard error of difference between the means.

Effects of group size on egg laying in the light:

Around 23 weeks of age, there was a difference due to group size on the consistency of hens laying in the light (Table 3-14). Hens in 4-bird cages laid proportionally fewer eggs in the light than birds in 2- and 8-bird cages.

Table 3-14 - The effects of group size on consistency of egg laying in the light, for birds in groups of 2, 4 and 8 per cage. Values shown are the angular transformed percentage means with back-transformed means in parentheses.

Group size treatments:	2 birds	4 birds	8 birds	SED	P Value
The 10 eggs recorded prior to:					
23 wks of age	82a (98%)	67b (85%)	83a (98%)	5.6	0.020
29 wks of age	67 (85%)	72 (91%)	74 (92%)	5.8	0.50
35 wks of age	80 (97%)	74 (92%)	77 (95%)	4.5	0.45

Within rows, means with different superscripts differ significantly P<0.05.
SED: standard error of difference between the means.

Effects of light schedule on egg laying in the light:

Following the 23-week data sampling period, the Modified light schedule was introduced for one-half of the birds, and there was a general decline in the proportion of eggs laid in the light in the Modified light treatment. However, there were only weak (P<0.1) differences due to the light schedule main effect on the proportion of eggs laid in the light around the 29 and 35 week of age data sampling periods (Table 3-15). As indicated in Table 3-15 and

Figure 3-17, most hens in the Standard light schedule treatment laid most, if not all, eggs during the light period.

Table 3-15 - The effects of light schedule on the proportion of eggs laid in the light. Values shown are the angular transformed percentage means with back-transformed means in parentheses.

Light schedule treatments:	Standard	Modified	SED	P Value
The 10 eggs recorded prior to:				
29 wks of age	87 (100%)	56 (68%)	4.5	0.092
35 wks of age	90 (100%)	64 (81%)	3.0	0.099

SED: standard error of difference between the means.
Note – the modified light schedule was imposed from 24 weeks of age; Light (L); Dark (D)
Modified light schedule: 13h L – 5h D – 3h L – 3h D
Standard light schedule: 16h L – 8h D

Egg laying in darkness:

Through modification of the light: dark schedule to introduce a 3-hour period of light from midnight to 03.00 h, a proportion of birds laid in the dark. Prior to the light modification at about 170 days of age, 9.3% of eggs were laid in the dark (grand mean for all hens). In the Standard light schedule treatment (16 h light followed by 8 hour dark per 24 hour), 50% of hens laid at least 1 egg in the dark compared to 57.8% of hens in the Modified light treatment (13 h L : 5 h D : 3 h L : 3 h D). Following the imposition of the Modified light schedule at 170 days, and after adjusting for the proportion of eggs laid in the dark prior to the change (ie. co-variate), 0.4% of eggs in the Standard light treatment were laid in the dark (10.7% of hens laid at least one egg in the dark) compared to 29.5% in the Modified light treatment (76.8% of hens laid at least 1 egg in the dark).

Thus, while the incidence of eggs laid in the dark was higher in the early stages of lay, the proportion declined with time. The application of the Modified light schedule treatment resulted in 25% of hens laying at least 50% of their eggs in the dark after 170 days of age, with 1 hen recorded to lay 100% of her eggs in the dark.

The distribution of hens according to the proportion of eggs they were recorded to lay in the dark during the experiment is shown in Figures 3-15 to 3-18. The data sets are presented according to the light schedule treatment imposed and the age of the birds.

Figures below: Egg laying in the dark by hens in the 2 light schedule treatments (Standard - solid bars (n=56) and Modified - open bars (n=56)). The data are distributed according to the proportion of eggs laid in the dark by each hen. The upper and lower pairs of graphs, respectively, represent the periods prior to and after 24 wks of age. The Modified light schedule was applied to 1 of 2 experimental rooms per replicate from 24 wks of age. Light: L, Dark: D.

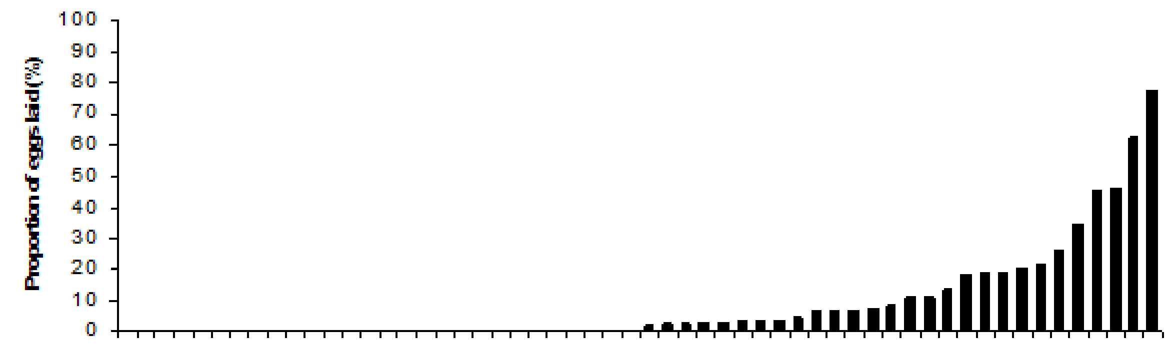


Figure 3-15- Standard light schedule pre-24 wks of age - 16 h L followed by (□) 8 h D

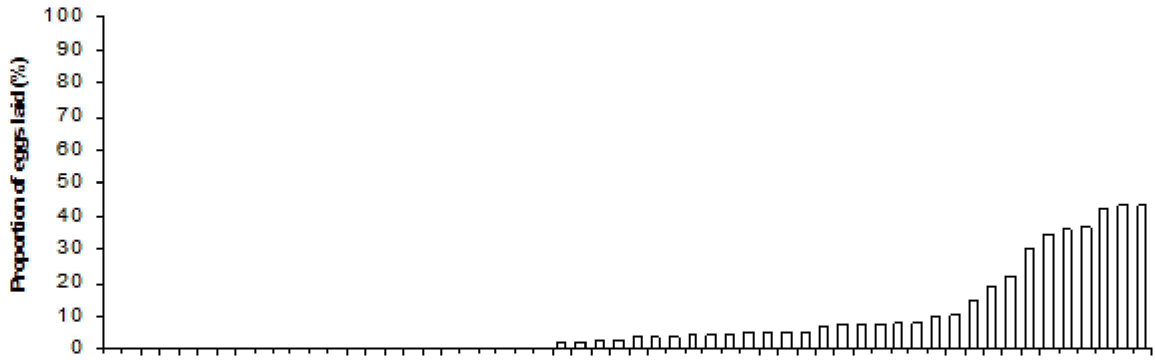


Figure 3-16 - Modified light schedule pre-24 wks of age - 16 h L □ 8 h D.

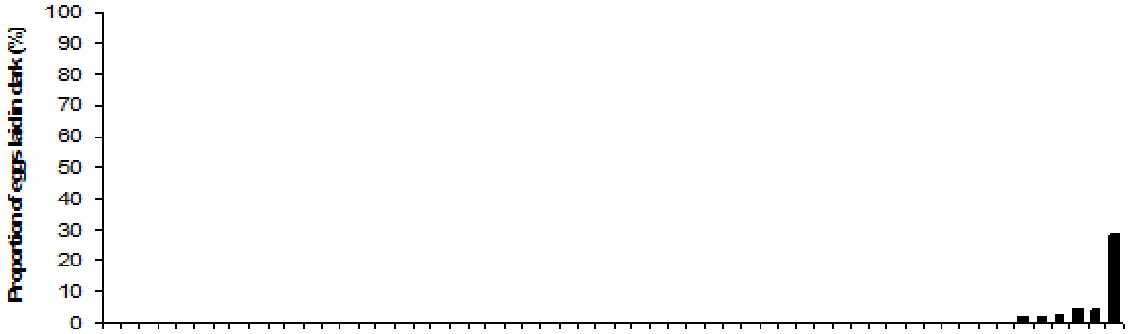


Figure 3-17 - Standard light schedule post-24 wks - 16 h L □ 8 h D.

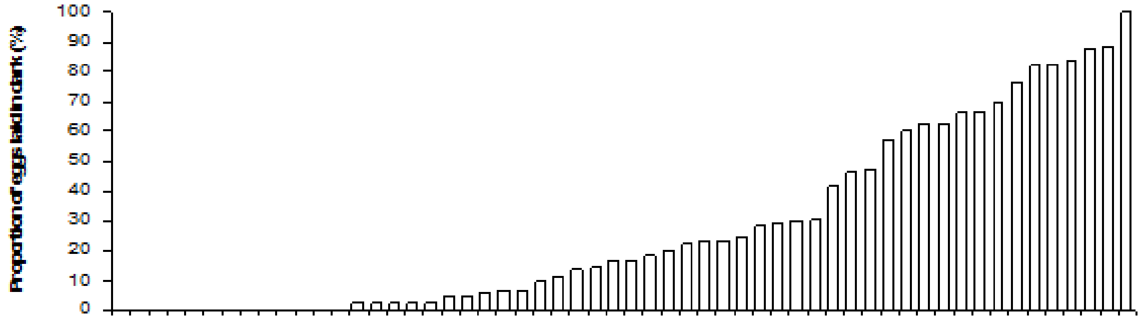


Figure 3-18 - Modified light schedule post-24 wks - 13 h L □ 5 h D □ 3 h L □ 3 h D.

3.4.4 Egg laying patterns – Timing and synchrony of oviposition

The spread of egg laying times for the 112 birds, based on cage averages over 4 quartiles, is shown in Figure 3-19. The 3 horizontal histograms in the figure show the quartile spread of egg laying times within 3 periods of the experiment, when the birds were (1) less than 24 weeks old, (2) between 24 and 30 weeks and (3) older than 30 weeks of age to the end of the experiment at 38 weeks. The inter-quartile width (period) is indicative of time synchrony. Narrower (smaller) quartiles indicate increased synchrony of egg laying times. As suggested in Figure 3-19, on average the 'middle' 50% of eggs (ie. quartiles 2 and 3) were laid within a relatively short time period (~2 h) each day.

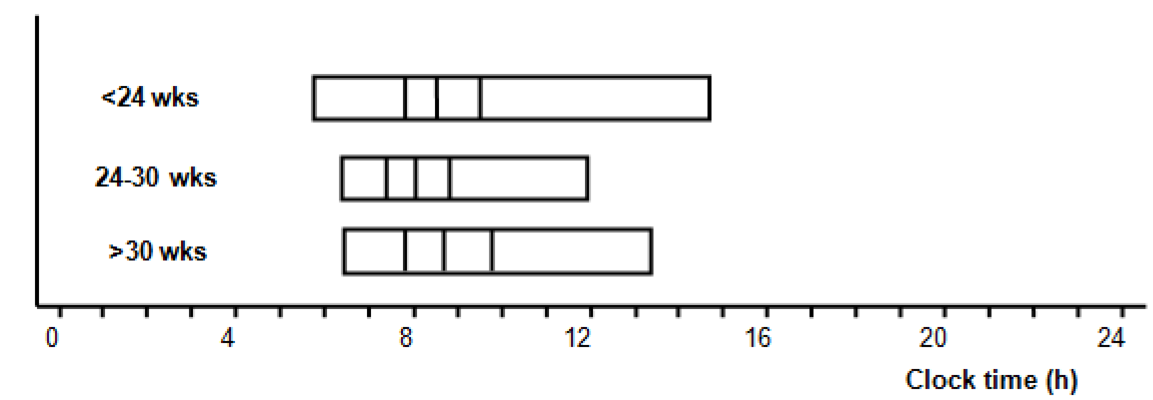


Figure 3-19 - Change in the quartile spread of egg-laying times by the 112 hens in experiment 1, in the period (1) prior to 24 weeks of age, (2) from 24 to 30 weeks of age and (3) after 30 weeks of age. The times shown are grand means derived from cage average values. Each quartile represents 25% of eggs laid.

3.4.4.1 The effects of the nest box on the timing and synchrony of oviposition:

The effects of the presence or absence of a nest box in the cage on the timing and synchrony of oviposition by hens as the experiment progressed, are shown in Figure 3-20 and Table 3-16. The quartile widths in Figure 3-20 are indicative of the synchrony of laying times. Prior to 24 weeks of age, the mean 50th and 75th percentile eggs were laid sooner per day in cages with compared to without a nest box (50th percentile time: 8.20 h vs. 8.80 h, SED 0.272, P=0.042; 75th percentile: 8.92 h vs. 9.83 h, SED 0.342, P=0.017). While significant time differences due to the nest box main effect were also found at 24-30 weeks of age, there were no differences detected after 30 weeks of age (Figure 3-20).

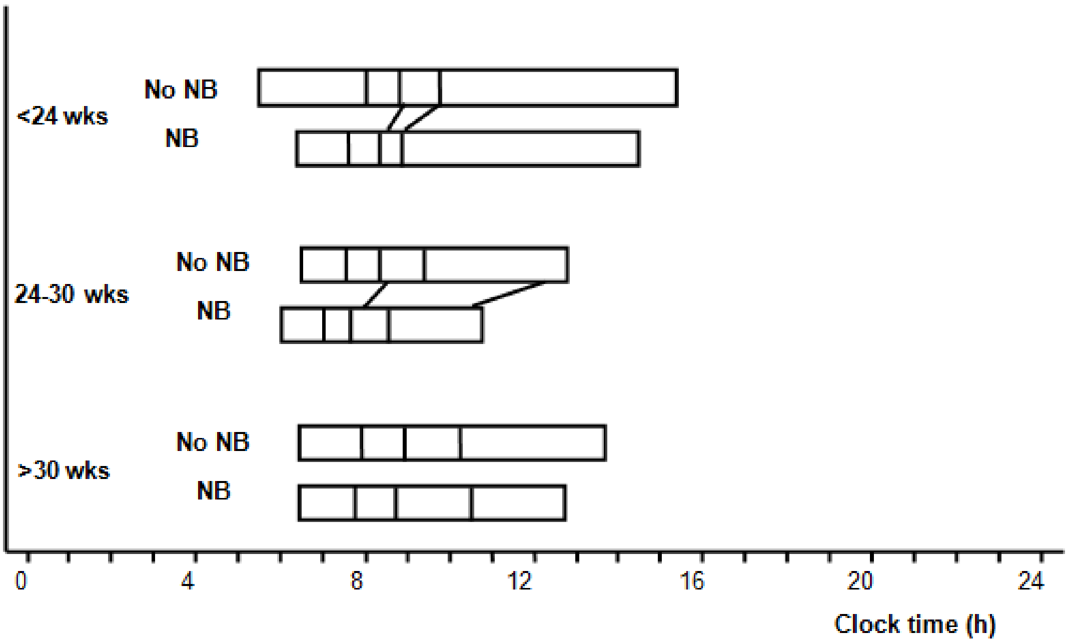


Figure 3-20 - The effects of a nest box (NB) or no nest box (No NB) in the cage on the quartile spread of egg-laying times, (1) prior to 24 wks of age, (2) from 24 to 30 wks and (3) after 30 wks. Within age profiles, oblique lines connecting No NB and NB quartiles indicate a significant difference between the values (P<0.05). The times shown are derived from cage average values.

Differences due to the nest box main effect on the inter-quartile period were found prior to 24 weeks of age, but not from 24 weeks onwards (Table 3-16).

Table 3-16 - The effects of presence or absence of a nest box in the cage on the synchrony of egg laying, represented by inter-quartile periods. The spread of egg laying is presented over 3 ages, prior to 24 wks, 24-30 wks and after 30 wks of age. Values shown h and based on cage averages.

Variate	Nest box	No Nest box	SED	P Value
< 24 wks old				
1st inter-quartile period	1.39	2.62	0.532	0.035
2nd inter-quartile period	0.61	0.76	0.087	0.096
3rd inter-quartile period	0.72	1.03	0.128	0.028
4th inter-quartile period	5.45	5.42	0.720	0.983
24-30 wks old				
1st inter-quartile period	0.95	1.14	0.172	0.295
2nd inter-quartile period	0.68	0.81	0.145	0.366
3rd inter-quartile period	0.90	1.02	0.143	0.448
4th inter-quartile period	2.30	3.50	0.646	0.092
> 30 wks old				
1st inter-quartile period	1.35	1.57	0.209	0.327
2nd inter-quartile period	0.91	0.98	0.190	0.737
3rd inter-quartile period	0.84	1.23	0.178	0.055
4th inter-quartile period	3.41	3.70	0.698	0.677

SED: standard error of difference between the means.

3.4.4.2 The effects of group size on the timing and synchrony of oviposition:

There were no differences due to group size on the timing of the first, 25th, 50th, 75th or last percentile egg laid (Table 3-17). However, as shown in Table 3-18, there were differences in the inter-quartile periods (synchrony) due to group size. For birds younger than 24 weeks, the second and third inter-quartile periods were longer for birds in 4- than 2- and 8-bird cages. Between 24 and 30 weeks of age, the first inter-quartile period was shorter in 2- compared to 4- and 8-bird cages.

Table 3-17- The effects of group size on the timing of egg laying, based on quartiles. Egg laying times are presented for 3 hen ages, prior to 24 wks, 24-30 wks and after 30 wks. Values shown are times in h and based on cage averages.

Group size treatments:	2 birds	4 birds	8 birds	SED	P Value
< 24 wks old					
Mean time of 1st egg in the day	5.88	5.50	6.07	0.684	0.710
First quartile time	7.86	7.60	7.99	0.343	0.536
Second quartile time	8.38	8.47	8.65	0.333	0.716
Third quartile time	9.07	9.60	9.45	0.419	0.455
4th quartile time	13.75	15.32	15.38	0.842	0.122
24- 30 wks old					
Mean time of 1st egg in the day	6.19	5.95	6.42	0.396	0.525
First quartile time	6.90	7.26	7.54	0.367	0.264
Second quartile time	7.68	8.00	8.26	0.303	0.209
Third quartile time	8.63	9.03	9.16	0.358	0.348
4th quartile time	11.21	12.31	12.00	0.806	0.400
> 30 wks old					
Mean time of first egg in the day	6.14	6.50	6.24	0.356	0.596
First quartile time	7.63	7.87	8.04	0.370	0.339
Second quartile time	8.62	8.73	8.88	0.459	0.852
Third quartile time	9.73	9.72	9.89	0.451	0.912
4th quartile time	12.91	13.75	13.34	0.595	0.402

SED: standard error of difference between the means.

Table 3-18 - The effects of group size on the inter-quartile period of egg laying. The spread of egg laying is presented over 3 ages, prior to 24 wks, 24-30 wks and after 30 wks of age. Values shown are h and based on cage averages.

Group size treatments:	2 birds	4 birds	8 birds	SED	P Value
< 24 wks old					
1st inter-quartile period	1.99	2.09	1.92	0.641	0.961
2nd inter-quartile period	0.52a	0.87b	0.66a	0.106	0.018
3rd inter-quartile period	0.70a	1.13b	0.80a	0.157	0.036
4th inter-quartile period	4.66	5.71	5.93	0.8818	0.331
24-30 wks old					
1st inter-quartile period	0.71a	1.30b	1.13b	0.211	0.048
2nd inter-quartile period	0.78	0.74	0.72	0.177	0.941
3rd inter-quartile period	0.95	1.03	0.90	0.175	0.733
4th inter-quartile period	2.57	3.29	2.83	0.792	0.676
> 30 wks old					
1st inter-quartile period	1.35	1.37	1.66	0.256	0.424
2nd inter-quartile period	1.14	0.86	0.84	0.198	0.392
3rd inter-quartile period	1.11	0.98	1.01	0.218	0.830
4th inter-quartile period	3.17	4.03	3.46	0.857	0.609

SED: standard error of difference between the means.
Within rows, means with different superscripts differ significantly $P<0.05$.

3.4.4.3 The effects of light schedule on the timing and synchrony of oviposition.

At entry to the experimental rooms, all birds were exposed to 12 h light followed by 12 h dark. After 2 weeks the component of light was gradually increased until birds received 16 h light and 8 h dark per day. In Figure 3-21 both cohorts of birds (treatments) are labelled 'Std' treatment prior to 24 weeks, indicating they received the same light schedule; there were no differences in the time of the first egg, the quartile times and the inter-quartile interval times (based on cage averages). The application of the Modified light schedule treatment, which included a period of light from midnight to 03.00 h, shifted egg laying times when birds were 24-30 weeks (Figure 3-21 and Table 3-19) and tended to reduce the synchrony of egg laying in the second quartile (P=0.063, Table 2-26). As reported previously in Figure 3-18, a proportion of birds responded to the light manipulation by laying eggs in the dark, between 03.00 and 06.00 h.

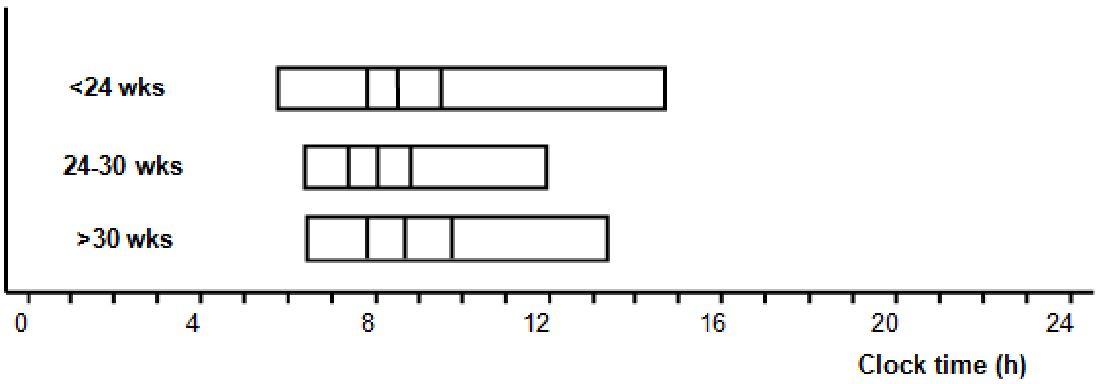


Figure 3-21- Change in the quartile spread of egg-laying times by the 112 hens in experiment 1, in the period (1) prior to 24 weeks of age, (2) from 24 to 30 weeks of age and (3) after 30 weeks of age. The times shown are grand means derived from cage average value

Differences due to the nest box main effect on the inter-quartile period were found prior to 24 weeks of age, but not from 24 weeks onwards (Table 3-19).

Table 3-19 - The effects of presence or absence of a nest box in the cage on the synchrony of egg laying, represented by inter-quartile periods. The spread of egg laying is presented over 3 ages, prior to 24 wks, 24-30 wks and after 30 wks of age. Values shown are h and based on cage averages

Variate	Nest box	No Nest box	SED	P Value
< 24 wks old				
1st inter-quartile period	1.39	2.62	0.532	0.035
2nd inter-quartile period	0.61	0.76	0.087	0.096
3rd inter-quartile period	0.72	1.03	0.128	0.028
4th inter-quartile period	5.45	5.42	0.720	0.983
24-30 wks old				
1st inter-quartile period	0.95	1.14	0.172	0.295
2nd inter-quartile period	0.68	0.81	0.145	0.366
3rd inter-quartile period	0.90	1.02	0.143	0.448
4th inter-quartile period	2.30	3.50	0.646	0.092
> 30 wks old				
1st inter-quartile period	1.35	1.57	0.209	0.327
2nd inter-quartile period	0.91	0.98	0.190	0.737
3rd inter-quartile period	0.84	1.23	0.178	0.055
4th inter-quartile period	3.41	3.70	0.698	0.677

SED: standard error of difference between the means

3.4.4.4 The effects of group size on the timing and synchrony of oviposition.

There were no differences due to group size on the timing of the first, 25th, 50th, 75th or last percentile egg laid (Table 3-20). However, as shown in Table 3-21, there were differences in the inter-quartile periods (synchrony) due to group size. For birds younger than 24 weeks, the second and third inter-quartile periods were longer for birds in 4- than 2- and 8-bird cages. Between 24 and 30 weeks of age, the first inter-quartile period was shorter in 2- compared to 4- and 8-bird cages.

Table 3-20 - The effects of group size on the timing of egg laying, based on quartiles. Egg laying times are presented for 3 hen ages, prior to 24 wks, 24-30 wks and after 30 wks. Values shown are times in h and based on cage averages.

Group size treatments:	2 birds	4 birds	8 birds	SED	P Value
< 24 wks old					
Mean time of 1st egg in the day	5.88	5.50	6.07	0.684	0.710
First quartile time	7.86	7.60	7.99	0.343	0.536
Second quartile time	8.38	8.47	8.65	0.333	0.716
Third quartile time	9.07	9.60	9.45	0.419	0.455
4th quartile time	13.75	15.32	15.38	0.842	0.122
24- 30 wks old					
Mean time of 1st egg in the day	6.19	5.95	6.42	0.396	0.525
First quartile time	6.90	7.26	7.54	0.367	0.264
Second quartile time	7.68	8.00	8.26	0.303	0.209
Third quartile time	8.63	9.03	9.16	0.358	0.348
4th quartile time	11.21	12.31	12.00	0.806	0.400
> 30 wks old					
Mean time of first egg in the day	6.14	6.50	6.24	0.356	0.596
First quartile time	7.63	7.87	8.04	0.370	0.339
Second quartile time	8.62	8.73	8.88	0.459	0.852
Third quartile time	9.73	9.72	9.89	0.451	0.912
4th quartile time	12.91	13.75	13.34	0.595	0.402

SED: standard error of difference between the means.

Table 3-21 - The effects of group size on the inter-quartile period of egg laying. The spread of egg laying is presented over 3 ages, prior to 24 wks, 24-30 wks and after 30 wks of age. Values shown are h and based on cage averages.

Group size treatments:	2 birds	4 birds	8 birds	SED	P Value
< 24 wks old					
1st inter-quartile period	1.99	2.09	1.92	0.641	0.961
2nd inter-quartile period	0.52a	0.87b	0.66a	0.106	0.018
3rd inter-quartile period	0.70a	1.13b	0.80a	0.157	0.036
4th inter-quartile period	4.66	5.71	5.93	0.8818	0.331
24-30 wks old					
1st inter-quartile period	0.71a	1.30b	1.13b	0.211	0.048
2nd inter-quartile period	0.78	0.74	0.72	0.177	0.941
3rd inter-quartile period	0.95	1.03	0.90	0.175	0.733
4th inter-quartile period	2.57	3.29	2.83	0.792	0.676
> 30 wks old					
1st inter-quartile period	1.35	1.37	1.66	0.256	0.424
2nd inter-quartile period	1.14	0.86	0.84	0.198	0.392
3rd inter-quartile period	1.11	0.98	1.01	0.218	0.830
4th inter-quartile period	3.17	4.03	3.46	0.857	0.609

SED: standard error of difference between the means.
Within rows, means with different superscripts differ significantly $P<0.05$.

3.4.4.5 The effects of light schedule on the timing and synchrony of oviposition.

At entry to the experimental rooms, all birds were exposed to 12 h light followed by 12 h dark. After 2 weeks the component of light was gradually increased until birds received 16 h light and 8 h dark per day. In Figure 3-22 both cohorts of birds (treatments) are labelled 'Std' treatment prior to 24 weeks, indicating they received the same light schedule; there were no differences in the time of the first egg, the quartile times and the inter-quartile interval times (based on cage averages). The application of the Modified light schedule treatment, which included a period of light from midnight to 03.00 h, shifted egg laying times when birds were 24-30 weeks (Figure 3-22 and Table 3-22) and tended to reduce the synchrony of egg laying in the second quartile (P=0.063, Table 3-23). As reported previously in Figure 3-18, a proportion of birds responded to the light manipulation by laying eggs in the dark, between 03.00 and 06.00 h.

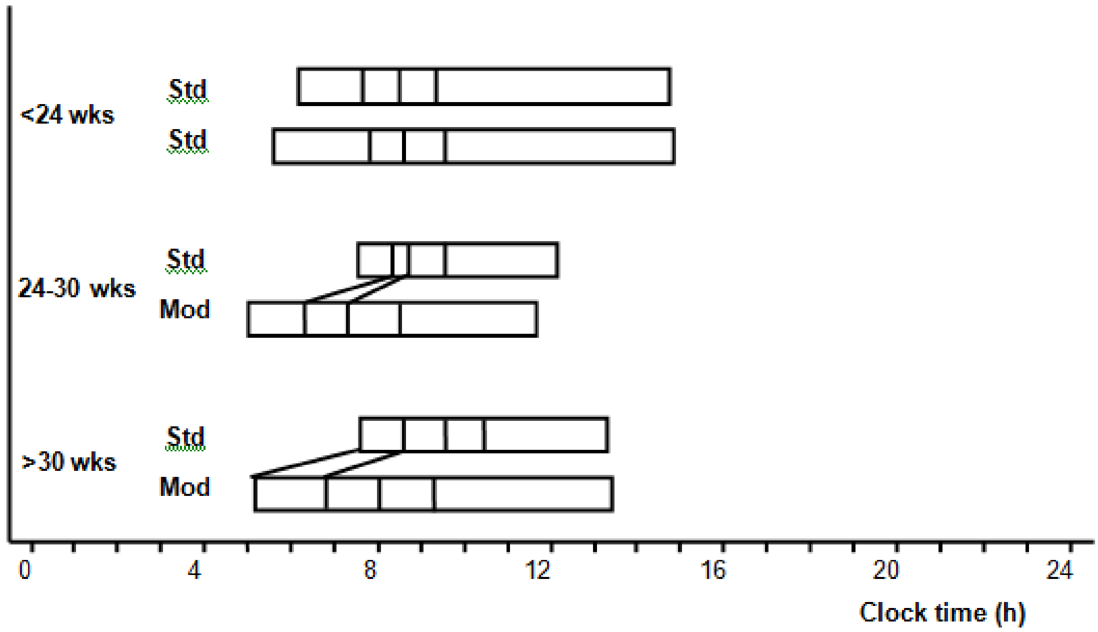


Figure 3-22 - The effects of light schedule (Standard (Std): 16h light (L)-8h dark (D) or Modified (Mod): 13h L-5h D-3h L-3h D) on the spread of egg-laying times at 3 ages, (1) prior to 24 wks, (2) from 24 to 30 wks and (3) after 30 wks. The Mod treatment was imposed from 24 wks. Within age profiles, obique lines connecting Std and Mod quartiles indicate a significant difference between the values (P<0.05). The times shown are derived from cage average values.

Table 3-22 - The effects of light schedule (Modified or Standard) on the timing of egg laying, based on quartiles. Egg laying times are presented for 3 hen ages, prior to 24 wks, 24-30 wks and after 30 wks. Values shown are h and based on cage averages.

Light schedule treatment:	Standard	Modified	SED	P Value
24- 30 wks old				
Mean time of 1st egg in the day	7.36	5.02	0.306	0.083
First quartile time	8.22	6.25	0.103	0.033
Second quartile time	8.73	7.23	0.056	0.024
Third quartile time	9.50	8.38	0.102	0.057
4th quartile time	12.05	11.64	0.816	0.696
> 30 wks old				
Mean time of 1st egg in the day	7.62	5.06	0.063	0.016
First quartile time	8.71	6.88	0.044	0.015
Second quartile time	9.45	8.04	0.422	0.184
Third quartile time	10.35	9.21	0.572	0.295
4th quartile time	13.25	13.42	1.023	0.886

SED: standard error of difference between the means.
Note – the modified light schedule was imposed from 24 weeks of age; Light (L); Dark (D)
Standard light schedule: 16h L – 8h D; Modified light schedule: 13h L – 5h D – 3h L – 3h D

Table 3-23- The effects of light schedule (Modified or Standard) on the synchrony of egg laying, estimated by the inter-quartile period, over 3 ages, prior to 24 wks, 24-30 wks and after 30 wks. Values shown are h and based on cage averages.

Light schedule treatment:	Standard	Modified	SED	P Value
24-30 wks old				
1st inter-quartile period	0.86	1.23	0.203	0.319
2nd inter-quartile period	0.51	0.98	0.046	0.063
3rd inter-quartile period	0.77	1.15	0.158	0.250
4th inter-quartile period	2.54	3.24	0.713	0.505
> 30 wks old				
1st inter-	1.10	1.82	0.108	0.094

quartile period				
2nd inter-quartile period	0.74	1.15	0.378	0.472
3rd inter-quartile period	0.90	1.17	0.150	0.322
4th inter-quartile period	2.88	4.22	0.454	0.209

SED: standard error of difference between the means.
Note – the modified light schedule was imposed from 24 weeks of age; Light (L); Dark (D)
Standard light schedule: 16h L – 8h D; Modified light schedule: 13h L – 5h D – 3h L – 3h D

3.4.4.6 Frequency of birds laying more than 1 egg per day.

Of the 112 hens in the experiment, 28 (25%) were recorded to lay more than 1 egg within a 'calendar' day on at least 1 occasion. The incidence of hens that did or did not lay multiple eggs per day is shown in Table 3-24.

Table 3-24 - Single and multiple eggs laid per day by hens. The table shows the distribution of hens that were only ever recorded to lay 1 egg per calendar day, or that were recorded to lay multiple eggs on 1 or more occasions.

Occasions when a hen laid >1 egg per day	0	1	2	3	4	5	6
Number of hens	84	18	5	3	0	1	1
Proportion of hens (%)			4.	2.	0.	0.	0.
	75.0	16.1	5	7	0	9	9

While hens in cages with a nest box were less likely ($P<0.05$) to lay multiple eggs in a day (16.1% and 33.9% of hens, respectively, for cages with and without a nest box; □21 = 4.76), there was no effect ($P>0.05$) of group size on this parameter (12.5%, 21.9% and 29.7% of hens, respectively, in groups of 2, 4 and 8 birds per cage; □22 = 2.244).

3.4.5 Welfare indicators

3.4.5.1 Measurement of plasma corticosterone concentrations.

Blood was collected between 13.00 and 14.00 h on 3 occasions from all hens for the measurement of plasma corticosterone concentrations. The pooled mean (±SD, minimum, maximum) times from oviposition to blood sampling were 296 min (±84.8, 99, 515) at 23 weeks, 299 min (±159, -99, 802) at 30 weeks and 201 min (±168, -365, 516) at 37 weeks. The pooled mean (±SD, minimum, maximum) times from capture of the bird to blood sampling were 99 s (±51, 25, 285) at 23 weeks, 91 s (±46, 13, 227) at 30 weeks and 94 s (±53, 19, 295) at 37 weeks. Data for a total of 2, 5 and 6 samples per sampling period, respectively, were omitted from the statistical analysis as the time from capture of the bird to blood sampling exceeded 180 s.

The data were analysed as cage average values (Tables 3-25, 3-26 and 3-27). Blood samples were collected between 1300-1500 h to avoid the anticipated period of pre-laying activity. Higher corticosterone concentrations are a response to higher stress levels.

Tables below: Plasma corticosterone concentrations in birds at 23, 30 and 37 weeks of age.

Table 3-25 - Nest box main effect (presence or absence of nest box in cage).

Nest box treatment:	Nest box	No Nest box	SED	P Value
23 wks (ng/mL)	4.36	3.28	0.253	<0.001
30 wks (ng/mL)	3.77	3.36	0.248	0.128
37 wks (ng/mL)	3.33	3.15	0.198	0.377

Table 3-26 - Group size main effect (birds per cage; in 8-bird cages).

Group size treatment:	2 birds	4 birds	8 birds	SED	P Value
23 wks (ng/mL)	3.76	3.75	3.95	0.309	0.769
30 wks (ng/mL)	3.32	3.55	3.82	0.303	0.306
37 wks (ng/mL)	2.72a	3.51b	3.49b	0.242	**0.015**

a, b Within rows, means with different superscripts differ significantly.

Table 3-27 - Light schedule main effect (1 or 2 light periods per 24 h period).

Light schedule treatment:	Standard	Modified	SED	P Value
30 wks (ng/mL)	3.55	3.58	0.019	0.323
37 wks (ng/mL)	3.27	3.21	0.164	0.782

SED: standard error of difference between the means
Values shown are cage means.
Note – the Modified light schedule was imposed from 24 weeks of age; Light (L); Dark (D)
Standard light schedule: 16h L – 8h D; Modified light schedule: 13h L – 5h D – 3h L – 3h D

Nest box main effect:

The data indicate differences due to the nest box main effect at 23 weeks of age. Corticosterone concentrations were 33% higher (P<0.001) in birds in cages with a nest box than those without a nest box (Table 3-25). There were no interactions between the main effects indicating the effect was due to the nest box per se, and not due to the effects of group size/space allowance in the cages, although it is recognised that group size/space allowance can affect stress responses in hens. However, at the subsequent 2 blood sampling occasions (30 and 37 weeks of age) there were no differences due to the nest box main effect and there were no interactions.

Group size main effect:

At the first and second blood sampling periods (23 and 30 weeks, respectively) there were no effects of group size and no interactions (Table 3-26). However, at the third sampling period (37 weeks of age), there was a difference due to group size. Cages with 2 birds had lower (P=0.015) plasma corticosterone concentrations than cages with 4 or 8 birds per cage. There were no interactions.

Light schedule main effect:

There were no differences due to the light schedule treatments on plasma corticosterone concentrations in the birds at 30 or 37 weeks of age (Table 3-27).

3.4.5.2 Response to ACTH challenge at 38 weeks of age

There were no differences due to main effects in plasma corticosterone concentrations following injection with ACTH, and there were no interactions (Tables 3-28, 3-29, 3-30).

Tables below: Plasma corticosterone concentrations in response to injection with ACTH at 38 weeks of age. Values shown are cage means.

Table 3-28 - Nest box main effect (presence or absence of nest box in cage).

Nest box treatment:	Nest box	No Nest box	SED	P Value
Response at 38 wks of age (ng/mL)	29.5	25.8	3.41	0.307

Table 3-29 - Group size main effect (birds per cage; in 8-bird cages).

Group size treatment:	2 birds	4 birds	8 birds	SED	P Value
Response at 38 wks of age (ng/mL)	25.6	28.4	29.0	4.17	0.695

Table 3-30 - Light schedule main effect (1 or 2 light periods per 24 h period).

Light schedule treatment:	Standard	Modified	SED	P Value
Response at 38 wks of age (ng/mL)	29.5	25.7	4.33	0.531

SED: standard error of difference between the means.
Note – the modified light schedule was imposed from 24 weeks of age; Light (L); Dark (D)
Standard light schedule: 16h L – 8h D; Modified light schedule: 13h L – 5h D – 3h L – 3h D

3.4.5.3 Measurement of egg albumen corticosterone concentrations

Corticosterone in egg albumen was assayed for the first 20 eggs laid per hen, as well as when the hens were aged 23, 29 and 35 weeks.

Egg albumen corticosterone concentrations measured over the first 20 eggs:

Figures 3-23 and 3-24 show the changes in mean egg albumen corticosterone concentrations over the first 20 eggs laid for the nest box and group size main effects, respectively. There were no significant differences in egg albumen corticosterone concentrations due to either main effect and there were no interactions. However, there were trends ($P<0.1$) for higher corticosterone concentrations in the second egg laid in cages with compared to without a nest box (1.09 and 0.94 ng/g, respectively; sed 0.083, $P=0.076$) and the tenth egg laid in 2- compared to 8-bird cages (cages with 2-, 4- and 8-birds 1.14, 0.99 and 0.95 ng/g, respectively; sed 0.085, $P=0.09$). Nevertheless, egg albumen corticosterone concentrations averaged over the first 20 eggs per bird laid did not differ due to either the nest box main effect (1.06 and 1.02 ng/g, respectively, for eggs laid in cages with and without a nest box, sed 0.039, $P=0.31$) nor the group size main effect (1.09, 1.03 and 1.00 ng/g, respectively, for eggs laid in cages with 2-, 4- or 8-birds, sed 0.048. $P=0.19$).

Figures below: Change in egg albumen corticosterone concentrations over the first 20 eggs laid.

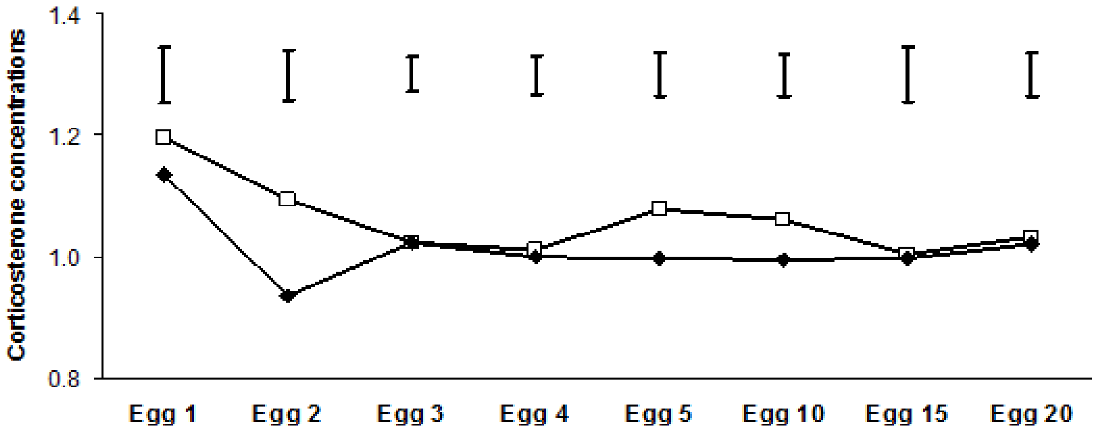


Figure 3-23 - Change in corticosterone concentrations (ng/g) in egg albumen over the first 20 eggs laid by hens in cages with (□) and without (•) a nest box. Bars represent standard error of difference. Values are based on cage means.

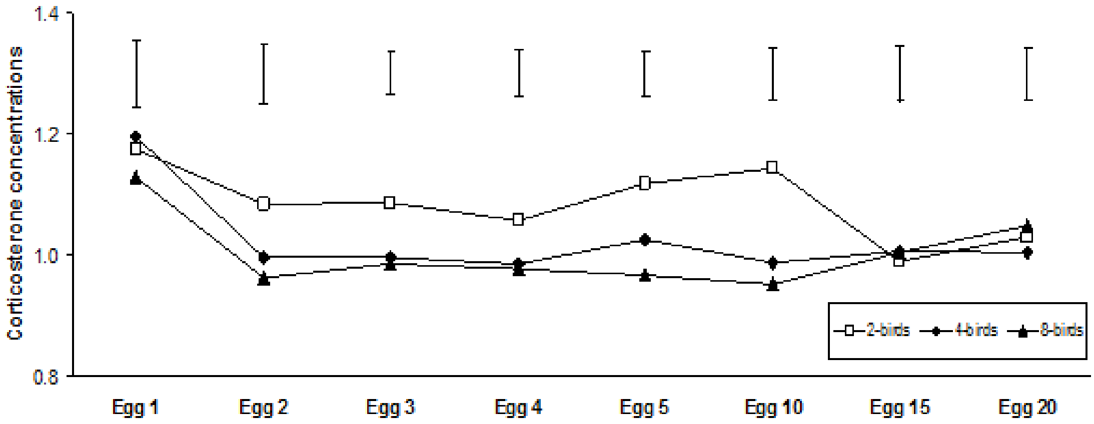


Figure 3-24 - Change in corticosterone concentrations (ng/g) in egg albumen over the first 20 eggs laid by hens in cages in groups of 2-, 4- and 8-birds per cage. Bars represent standard error of difference. Values are based on cage means.

Egg albumen corticosterone concentrations at 23, 29 and 35 weeks of age:

As shown in Tables 3-31, 3-32 and 3-33, there were no significant differences due to the main effects, and there were no interactions, on corticosterone concentrations in albumen of eggs collected from the birds at 23, 29 and 35 weeks of age.

Tables below: The effects of the treatments on corticosterone in egg albumen when birds were aged 23, 29 and 35 weeks.

Table 3-31 - The effects of presence or absence of a nest box in the cage on corticosterone concentrations (ng/g) in egg albumen.

Nest box treatment:	Nest box	No Nest box	SED	P Value
23 wks	1.03	1.08	0.075	0.48
29 wks	0.97	1.04	0.069	0.37
35 wks	0.96	0.97	0.046	0.85

Table 3-32 - The effects of group size on corticosterone concentrations (ng/g) in egg albumen.

Group size treatment:	2 birds	4 birds	8 birds	SED	P Value
23 wks	0.98	1.14	1.04	0.075	0.25
29 wks	0.95	0.97	1.09	0.084	0.27
35 wks	0.97	0.94	1.00	0.056	0.58

Table 3-33 - The effects of light-dark schedule on corticosterone concentrations (ng/g) in egg albumen.

Light schedule treatment:	Modified	Standard	SED	P Value
29 wks	1.01	1.00	0.062	0.83
35 wks	0.93	1.01	0.052	0.38

SED: standard error of difference between the means.
Note – the modified light schedule was imposed from 24 weeks of age; Light (L), Dark (D)
Standard light schedule: 16h L – 8h D; Modified light schedule: 13h L – 5h D – 3h L – 3h D

3.4.5.4 Measurement of blood haematology parameters

Heterophils and lymphocytes are the main blood cell types related to the stress response. The other white cell types are mostly related to allergy responses and responses to parasite infestations. The data in Tables 3-34, 3-35 and 3-36 show that, in general, there were no differences due to the nest box, group size or light schedule main effects on the haematological parameters. While there was a difference in absolute white blood cell count due to the light schedule main effect at 36 weeks of age (P=0.047), this was possibly due to a transient, minor health issue for the birds in one room (the light treatments were necessarily in separate rooms). Elevated total white blood cell count is not generally associated with a stress response.

Tables below: The effects of the treatments on blood haematology parameters in birds at 24, 31 and 36 weeks of age. The values shown are cage averages following log10 transformation. Values in parentheses are the back-transformed means.

Table 3-34 - The effects of a nest box in the cage on haematology parameters.

Nest box treatment	Hen age	Nest box	No Nest box	SED	P Value
White blood cell count (106/mL)	24 wks	1.22 (16.4)	1.24 (17.2)	0.044	0.65
	31 wks	1.19 (15.5)	1.22 (16.6)	0.049	0.54
	36 wks	1.18 (15.2)	1.17 (14.9)	0.085	0.85
Heterophil count (106/mL)	24 wks	0.91 (8.2)	0.92 (8.3)	0.064	0.96
	31 wks	1.00 (10.1)	1.04 (11.0)	0.048	0.43
	36 wks	1.00 (9.9)	0.98 (9.5)	0.041	0.65
Lymphocyte count (106/mL)	24 wks	0.86 (7.3)	0.90 (8.0)	0.053	0.47
	31 wks	0.63 (4.3)	0.57 (3.7)	0.099	0.55
	36 wks	0.48 (3.0)	0.44 (2.8)	0.084	0.65
Heterophil : Lymphocyte ratio	24 wks	0.20 (1.57)	0.21 (1.63)	0.131	0.91
	31 wks	0.47 (2.94)	0.59 (3.85)	0.103	0.29
	36 wks	0.57 (3.72)	0.62 (4.12)	0.065	0.50

SED: standard error of difference between the means

Table 3-35 - The effects group size on haematology parameters.

Group size treatment:	Hen age	2 birds	4 birds	8 birds	SED	P Value
White blood cell count (106/mL)	24 wks	1.21 (16.3)	1.23 (17.1)	1.23 (17.0)	0.054	0.91
	31 wks	1.18 (15.1)	1.25 (17.8)	1.19 (15.4)	0.060	0.46
	36 wks	1.20 (15.8)	1.19 (15.5)	1.14 (13.8)	0.060	0.60
Heterophil count (106/mL)	24 wks	0.89 (7.8)	0.96 (9.0)	0.90 (7.9)	0.078	0.67
	31 wks	1.01 (10.1)	1.08 (12.0)	0.99 (9.7)	0.58	0.30
	36 wks	0.99 (9.8)	1.02 (10.5)	0.95 (8.9)	0.050	0.37
Lymphocyte count (106/mL)	24 wks	0.87 (7.4)	0.85 (7.0)	0.93 (8.5)	0.064	0.44
	31 wks	0.60 (4.0)	0.61 (4.1)	0.59 (3.9)	0.122	0.98
	36 wks	0.45 (2.8)	0.49 (3.1)	0.44 (2.8)	0.103	0.85
Heterophil : Lymphocyte ratio	24 wks	0.19 (1.54)	0.29 (1.94)	0.14 (1.37)	0.160	0.91
	31 wks	0.47 (3.0)	0.60 (4.0)	0.51 (3.2)	0.127	0.58
	36 wks	0.59 (3.8)	0.61 (4.0)	0.59 (3.9)	0.080	0.96

SED: standard error of difference between the means

Table 3-36 - The effects of light schedule on haematology parameters.

Light schedule treatment:		Modified	Standard	SED	P Value
White blood cell count (106/mL)	31 wks	1.18 (15.2)	1.23 (16.9)	0.036	0.42
	36 wks	1.06 (11.5)	1.29 (19.6)	0.017	0.047
Heterophil count (106/mL)	31 wks	1.00 (9.9)	1.05 (11.3)	0.025	0.26
	36 wks	0.90 (7.9)	1.08 (11.9)	0.061	0.21
Lymphocyte count (106/mL)	31 wks	0.58 (3.8)	0.63 (4.2)	0.043	0.46
	36 wks	0.36 (2.3)	0.56 (3.6)	0.124	0.36
Heterophil : Lymphocyte ratio	31 wks	0.53 (3.4)	0.52 (3.3)	0.044	0.81
	36 wks	0.57 (3.7)	0.62 (4.1)	0.166	0.83

SED: standard error of difference between the means
Note – the modified light schedule was imposed from 24 weeks of age; Light (L); Dark (D)
Modified light schedule: 13h L – 5h D – 3h L – 3h D
Standard light schedule: 16h L – 8h D

3.4.6 The relationships between welfare indicators and egg laying characteristics

The majority of the data presented thus far in this final report are based on cage averages, that is the mean response for all birds in a cage. While the use of the cage of birds as the experimental unit is correct and robust in an analysis of variance to determine statistical differences due to the main effects or interactions, it does not allow the study of the responses of individual birds, potentially masking production or welfare problems of individual birds (Cunninghan et al. 1987). Within a group (cage) of birds, the individuals are organised in a social hierarchy and dominant birds may behave differently to subordinate individuals. For example, Cunningham et al. (1987) reported that subordinate birds at high stocking densities (<400 cm^2 / bird) in groups of 6-7 per cage had poorer egg production than dominant birds. A hen's position in the social hierarchy can alter access to resources (e.g. food, nest box, etc.) and perhaps how the resources in the environment are utilised. Similarly, as the data indicate, not all birds with access to a nest box used the nest box for egg laying. These and other issues could impact on the birds' ability to cope in a challenging environment, and thus may be relevant information for understanding the relationships between nest boxes and the welfare of laying hens in cages.

The observational methods applied to this experiment enabled the recognition of individual birds in groups and the recording of individual egg-laying characteristics. For example, although about 70% of eggs were laid in the nest box (Figure 3-2), the observational methodology enabled identification of birds based on their consistency of nest box use. Further, through the use of REML analysis (GenStat 2000), the egg-laying patterns of nest box and non-nest-box layers could be thus related to the various welfare indicators in the different data sampling periods.

3.4.6.1 Plasma corticosterone concentrations and egg laying.

Egg laid today or no egg today and plasma corticosterone concentrations:

Blood samples were taken from all birds in the 3 sampling periods, and using and the observation methodology and video technology, it was possible to identify which birds had or had not laid an egg on the days of blood sampling. There were no relationships between corticosterone concentrations in plasma collected between 13.00 and 15.00 h and whether or not the bird had laid an egg before the blood sample was taken that day.

Table 3-37 - The relationship between whether the hen laid an egg on the day of blood sampling and her plasma corticosterone concentrations (ng/mL) at 3 ages. The results are adjusted for the fixed effects of replicate, room within replicate and random effect of cage within room and replicate. The values shown are back-transformed means.

Hen age (wks)	Plasma corticosterone concentrations (ng/mL)	% increase in plasma corticosterone concentrations if no egg was laid	95% CI of % increase	P Value	
	No egg laid	Egg was laid			
23	3.72	3.65	-2	(-14, +12)	0.77
30	3.25	3.48	7	(-12, +31)	0.51
37	3.09	3.12	1	(-14, +18)	0.93

CI: confidence interval.

Interval from oviposition to blood sampling and plasma corticosterone concentrations:

The relationship between the interval from egg laying to blood sampling was calculated to determine whether hens that had recently laid an egg (or were about to lay when the blood sample was taken) showed higher plasma corticosterone concentrations. As shown in the series of graphs in the figures below, there were no significant relationships between these parameters at 23, 30 or 37 weeks of age.

Figures 3-26 and 3-27 show that blood samples were taken before egg laying occurred for 4 and 10 hens, respectively, at the 30 and 37 week sampling periods. At 30 weeks there were equal numbers of birds in cages with and without a nest box; the 2 birds in cages with a nest box were 100% nest-box layers. At 37 weeks there were 3 birds in the nest box treatment; 2 birds were 100% nest box layers and the other bird was a 100% floor layer.

Figures below: Relationships between the interval from egg laying to blood sampling and plasma corticosterone concentrations at 3 ages, adjusted for replicate, room and cage on the logarithmic scale.

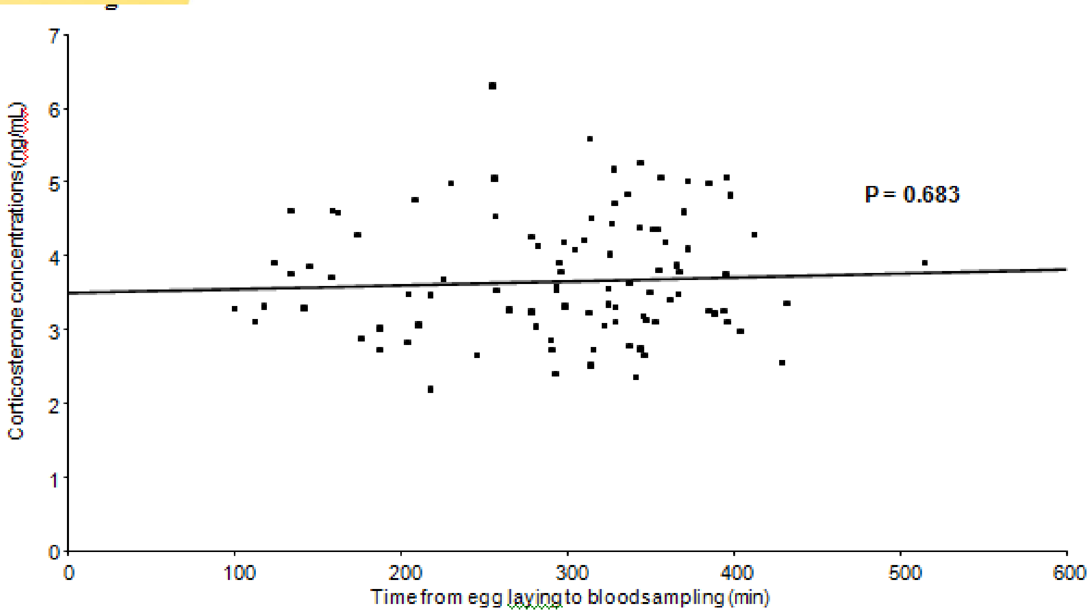


Figure 3-25 - The relationship between plasma corticosterone concentrations in blood samples at 23 weeks of age and time interval between oviposition and blood sampling.

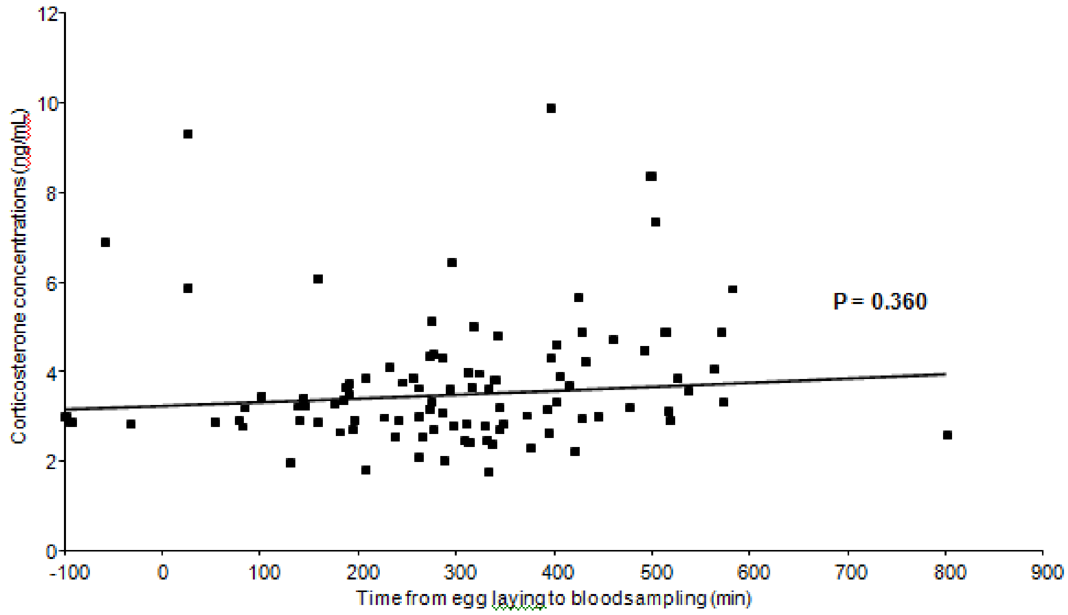


Figure 3-26 - The relationship between plasma corticosterone concentrations in blood samples at 30 weeks of age and time interval between oviposition and blood sampling. Negative values on the x-axis represent eggs laid after the blood sample was taken.

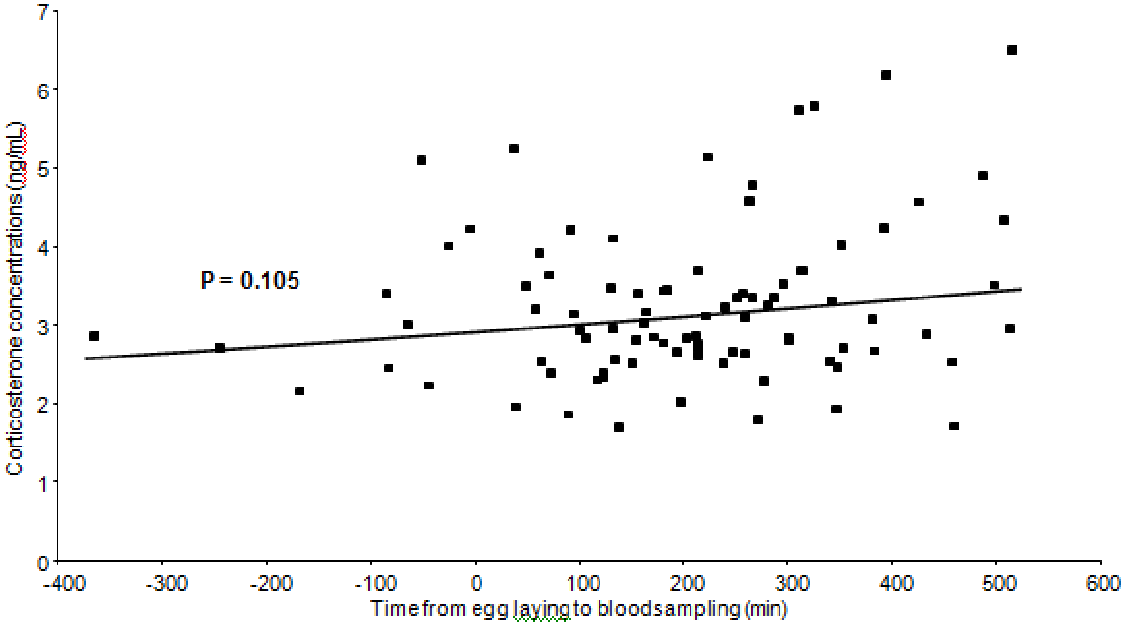


Figure 3-27 - The relationship between plasma corticosterone concentrations in blood samples at 37 weeks of age and time interval between oviposition and blood sample. Negative values on the x-axis represent eggs laid after the blood sample was taken.

3.4.6.2 The relationships between welfare parameters and consistency of egg laying in a nest box

Plasma corticosterone concentrations and consistency of nest box use:

There were no significant relationships between the consistency of nest box use (based on 10 sequential eggs recorded prior to the blood sample) and plasma corticosterone concentrations in blood samples collected between 1300 and 1500 h at either 23, 30 or 37 weeks of age (Figures 3-28, 3-29 and 3-30). Also, as shown in Figure 3-31, there was no relationship between the consistency of nest box use and plasma corticosterone response one hour after intra-muscular ACTH injection.

Figures 3-28, 3-29 and 3-30 below: The relationships between consistency of egg laying in the nest box (based on the previous 10 eggs) and plasma corticosterone concentrations at 3 ages.

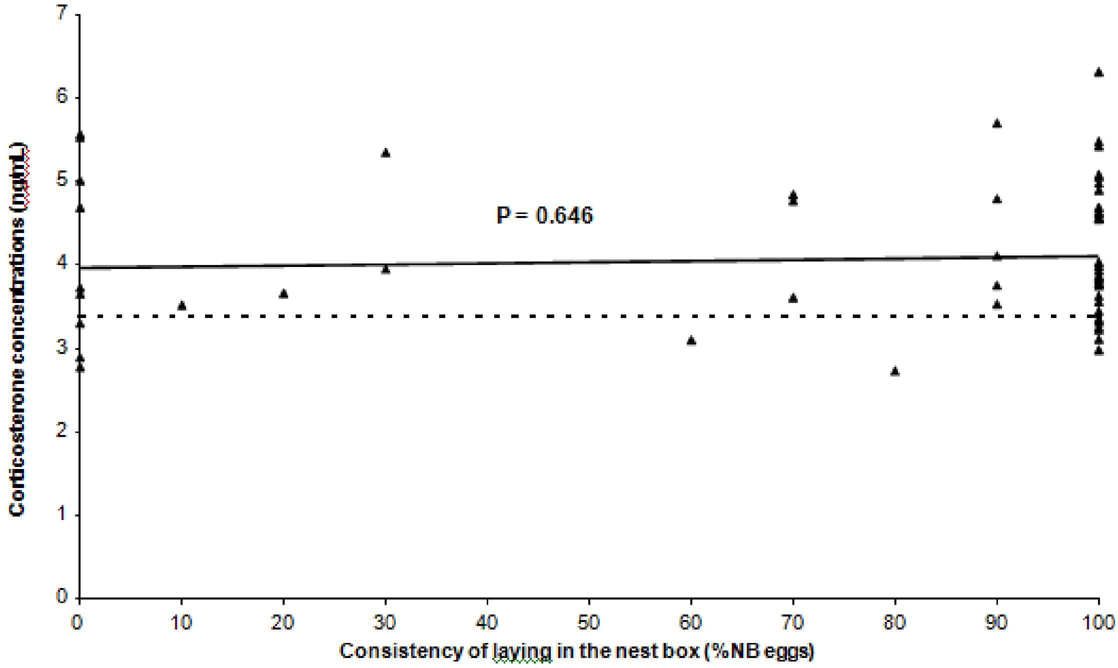


Figure 3-28 - The relationship between consistency of laying in the nest box and plasma corticosterone concentrations at 23 weeks of age (n=56 hens). The triangles are the individual data points for each bird. The upper (solid) line is the regression line between the parameters. The lower (dashed) line indicates the mean plasma corticosterone concentrations for hens in cages without a nest box at the same age.

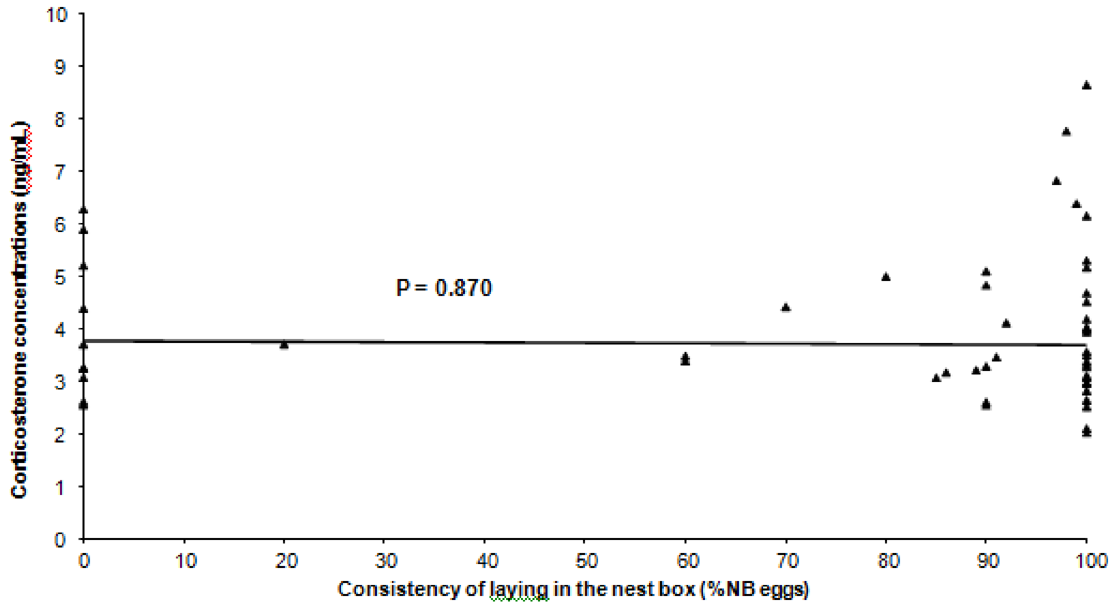


Figure 3-29 - The relationship between consistency of laying in the nest box and plasma corticosterone concentrations for birds at 30 weeks of age (n=56 hens). The triangles are the individual data points and the solid line is the regression line between the parameters.

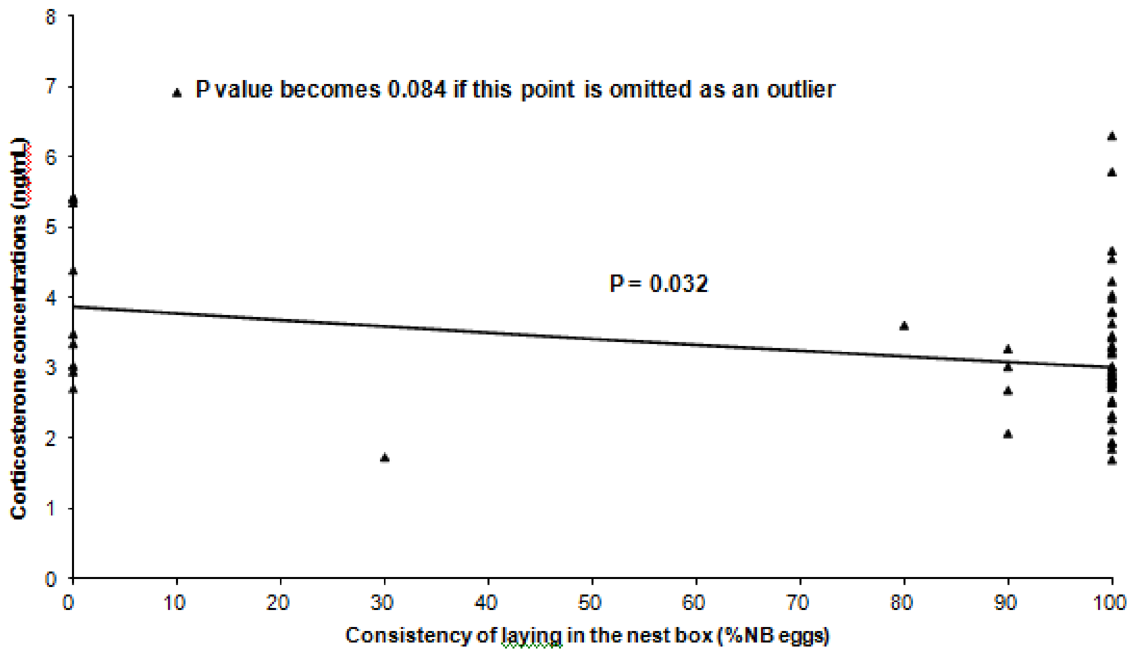


Figure 3-30 - The relationship between consistency of laying in the nest box and plasma corticosterone concentrations for birds at 37 weeks of age (n=56 hens). One data point in the top left of the distribution had a strong influence on the slope of the relationship. If the data point was omitted, the relationship changed and the probability became P=0.084.

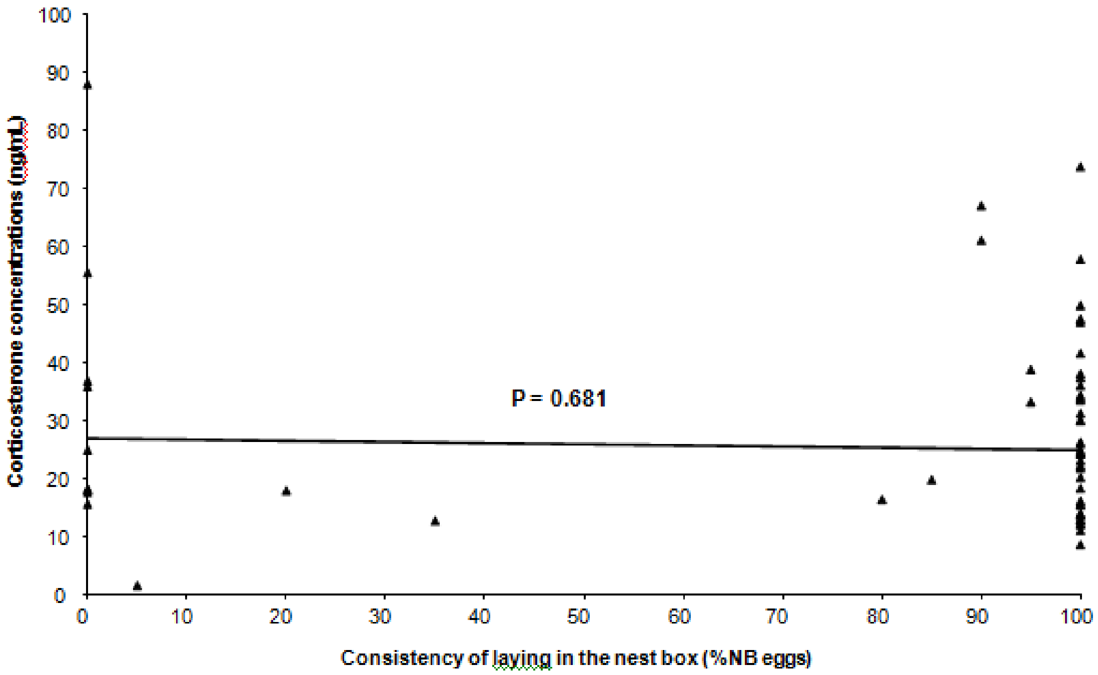


Figure 3-31 - The relationship between consistency of laying in the nest box over the previous 10 eggs, for hens in cages with a nest box, and plasma corticosterone concentrations in response to an intra-muscular injection of ACTH at 38 weeks of age (n=56 hens).

Egg albumen corticosterone concentrations and consistency of nest box use:

While there was no relationship between consistency of egg laying in the nest box over the first 10 eggs laid and the average egg albumen corticosterone concentrations in the 5th and 10th eggs (Figure 3-32), there was a highly significant quadratic relationship at 23 weeks of age (Figure 3-33).

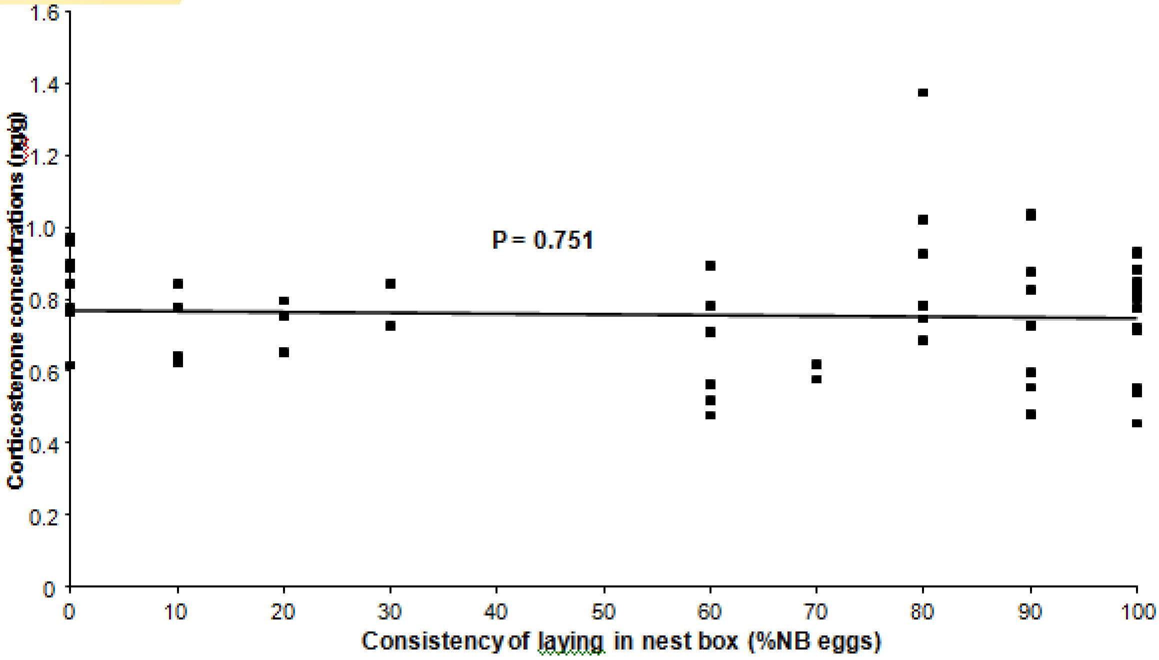


Figure 3-32 - The relationship between consistency of nest box use over the first 10 eggs laid (cages with nest box only) and mean corticosterone concentrations in the albumen (ng/g) of the 5th and 10th eggs laid. Concentrations were adjusted for replicate, room and cage and analysed after logarithmic transformation.

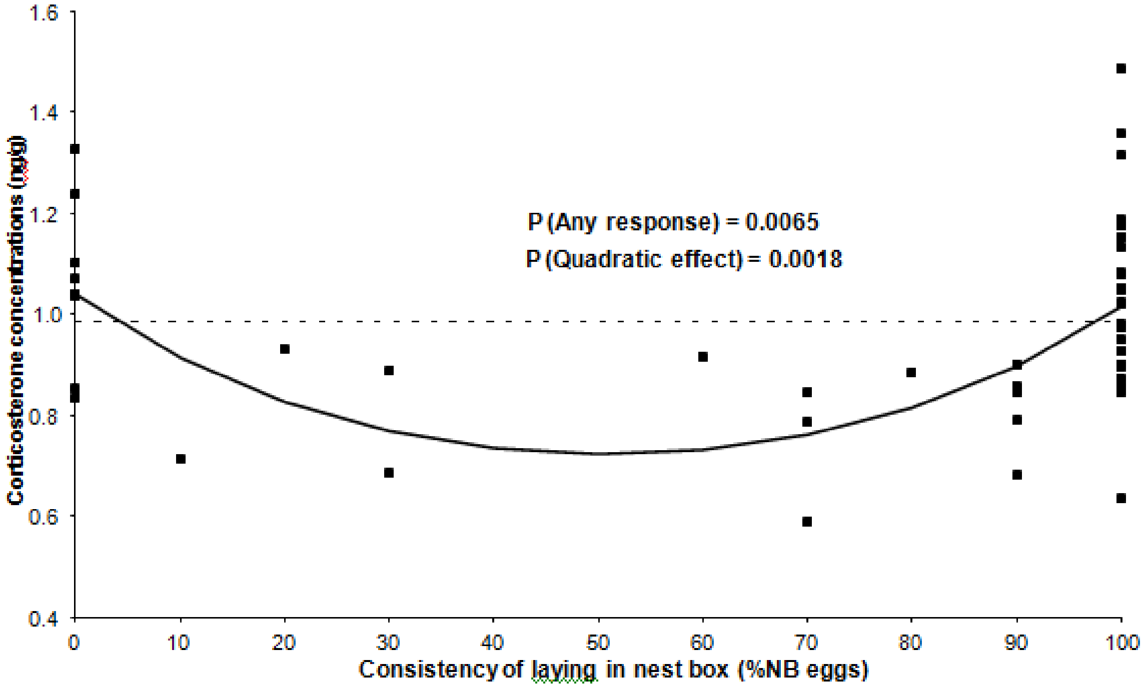
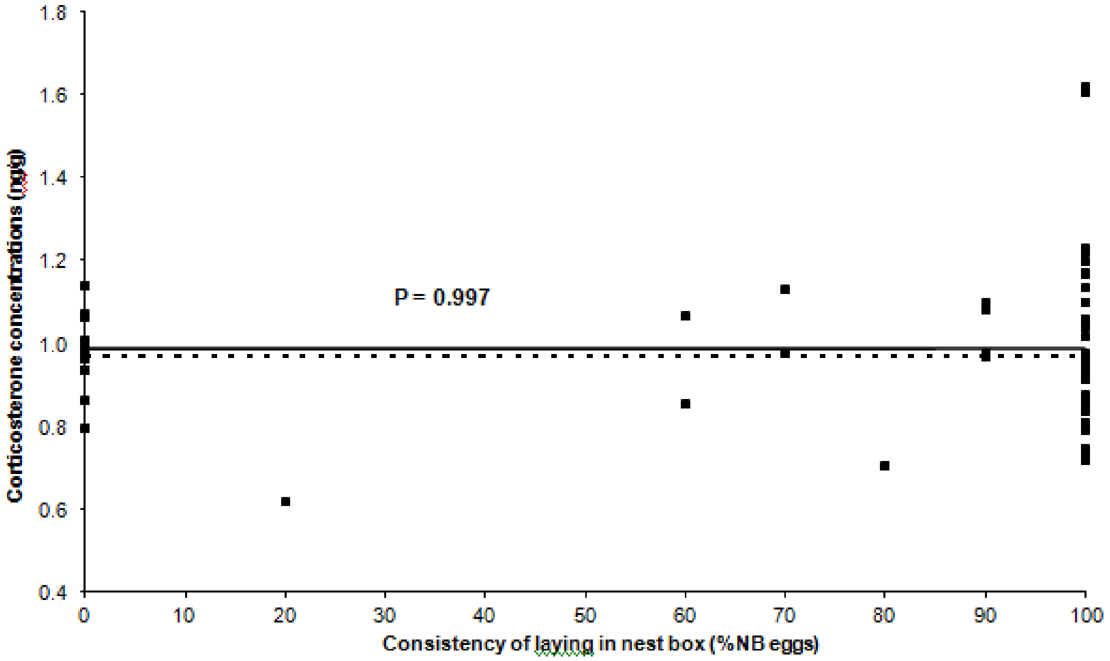


Figure 3-33 - The relationship between consistency of nest box use (previous 10 eggs) at 23 weeks of age (cages with nest box only) and egg albumen corticosterone concentrations (ng/g). Concentrations were adjusted for replicate, room and cage and analysed after logarithmic transformation. The horizontal (dashed) line shows the median egg albumen corticosterone concentrations in eggs from hens in cages without a nest box.

However, at the subsequent 2 data sampling periods there were no relationships between the parameters (Figure 3-34).



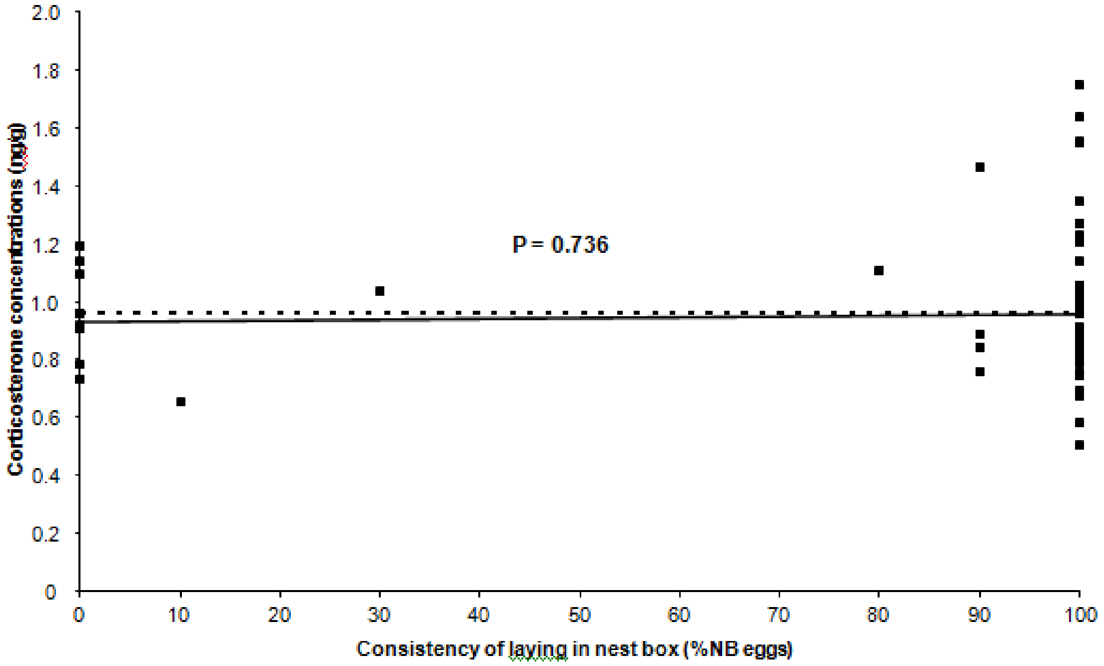


Figure 3-34 - Relationships between consistency of nest box use by hens at 29 (upper graph) and 35 (lower graph) weeks of age and egg albumen corticosterone concentrations (ng/g). Corticosterone concentrations were adjusted for replicate, room and cage and analysed after logarithmic transformation. The horizontal (dashed) lines show the median egg albumen corticosterone concentrations in eggs from hens in cages without a nest box at the 2 hen ages.

Heterophil to lymphocyte (H:L) ratio and consistency of nest box use:

The consistency of laying in the nest box was not related to the heterophil to lymphocyte ratio at any of the 3 sampling periods (Figure 3-35).

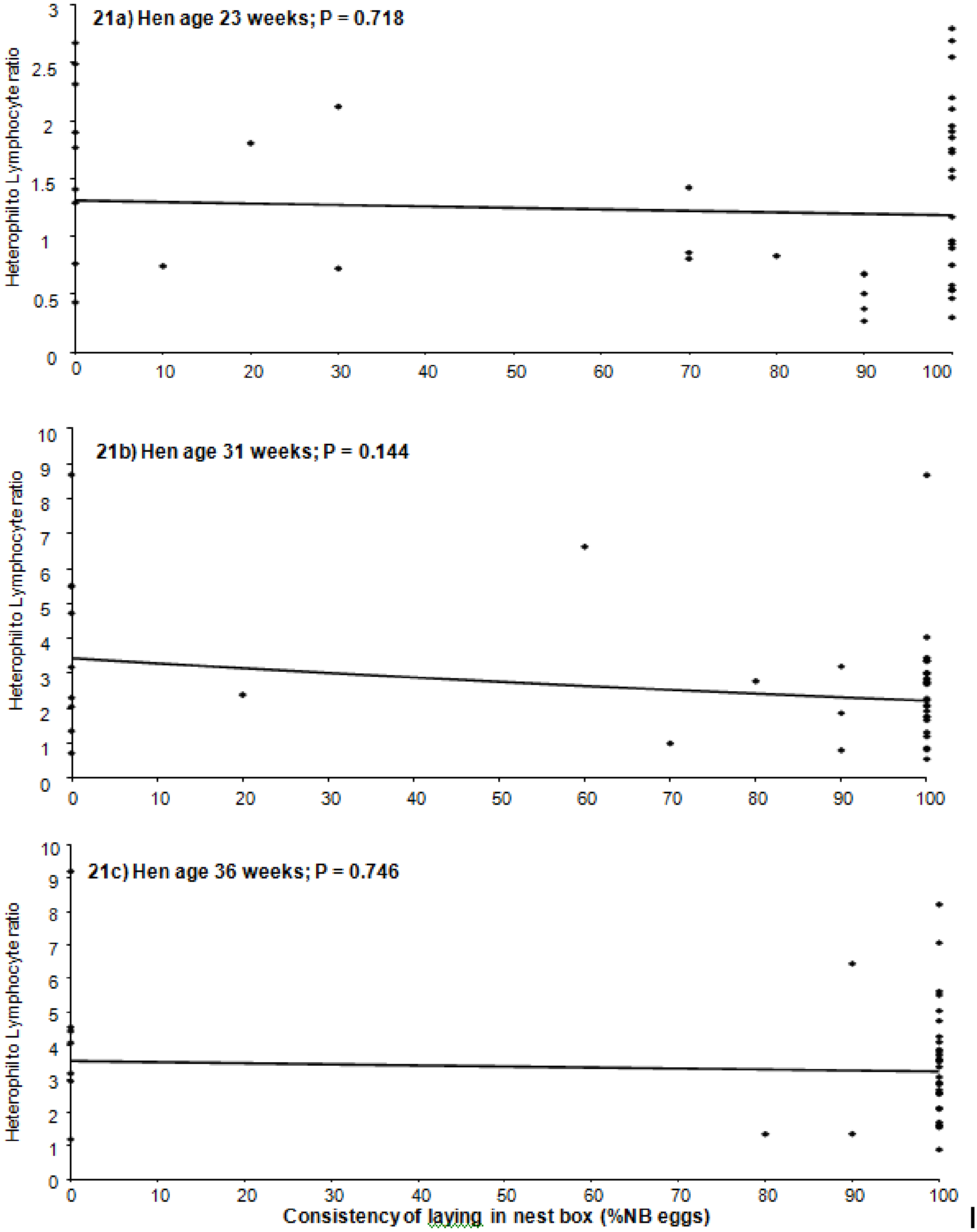


Figure 3-35 - Relationship between heterophil to lymphocyte ratio and the consistency of egg laying in the nest box for birds at 24, 31 and 36 weeks of age (cages with nest box only).

3.4.6.3 Consistency of egg-laying site and plasma corticosterone concentrations

The consistency of egg laying site was estimated for the 112 individual hens in the experiment using Pearson's goodness of fit statistic, based on a 5-zone distribution within the cage. Using this method it was possible to compare the consistency of egg-laying sites irrespective of whether birds came from cages with or without a nest box, and physiological stress response as indicated by plasma corticosterone. On a cage average basis, plasma corticosterone concentrations were found to differ due to the main effects in 2 situations:

1) at 23 weeks of age, plasma corticosterone was higher in cages with a nest box, and
2) at 37 weeks of age, plasma corticosterone was lower in cages with 2 birds.

The relationships between egg-laying site consistency and plasma corticosterone concentrations were therefore investigated.

Presence or absence of the nest box in the cage on the relationship between consistency of egg-laying site and plasma corticosterone concentrations:

There were no relationships between the consistency of egg-laying site, regardless of whether hens had access to a nest box or the proportion of eggs laid in the nest box or on the wire floor, and plasma corticosterone concentrations at 23, 30 and 37 weeks of age. The relationship between egg-laying site consistency score and plasma corticosterone concentrations at 23 weeks is shown in Figure 3-36. At 23 weeks of age it was found that hens in cages with a nest box had 33% higher plasma corticosterone concentrations than hens in cages without a nest box (based on cage averages). In Figure 3-36 this difference is represented by the solid fitted line above the dashed line.

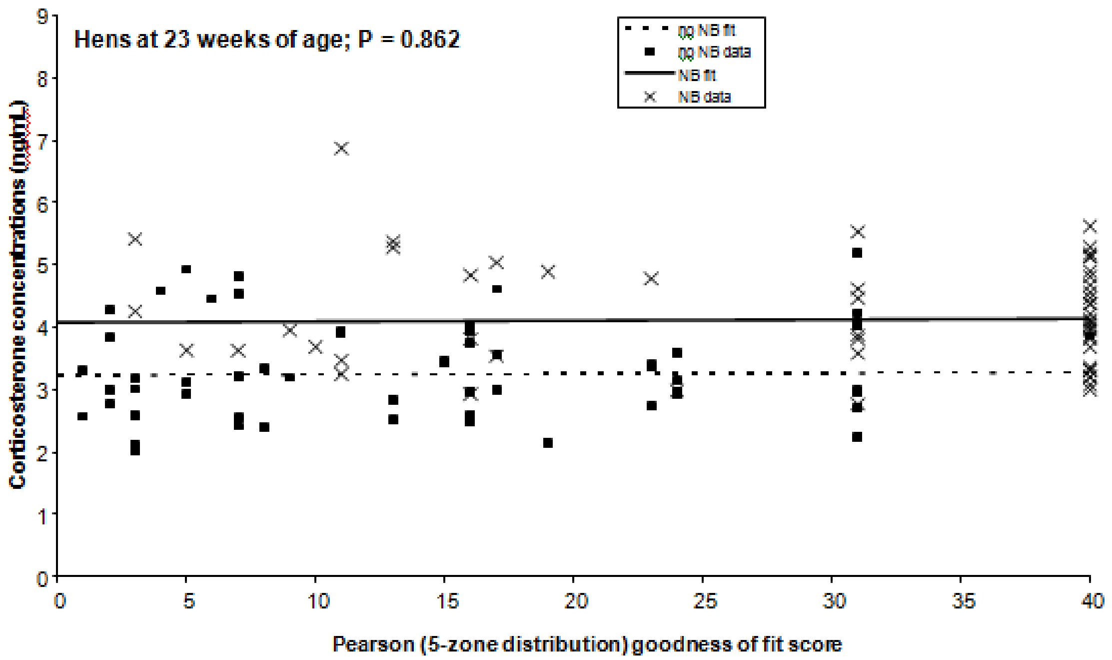


Figure 3-36 - Comparison of birds at 23 weeks of age in cages with and without a nest box (NB; n=112). The figure shows the relationship between consistency of egg-laying site based on a 5-zone Pearson's goodness of fit score and plasma corticosterone concentrations (ng/mL) adjusted for replicate, room and cage on the logarithmic scale. The solid line (upper) represents the mean for hens in cages with a nest box and the dashed line (lower) represents the mean for hens in cages without a nest box. The 'x' and ■ symbols represent the data points for individual hens from cages with and without a nest box, respectively.

The effect of group size on the relationship between consistency of egg-laying site and plasma corticosterone concentrations:

At 37 weeks of age the results indicated (based on cage averages) that birds in 2-bird compared to 4- and 8-bird cages has lower plasma corticosterone concentrations. Figure 2-33 however, shows there was no relationship between consistency of egg-laying site by hens (whether in the nest box or on the wire floor) and plasma corticosterone, regardless of number of birds per cage. Similar non-significant relationships were found for birds at 23 and 30 weeks of age.

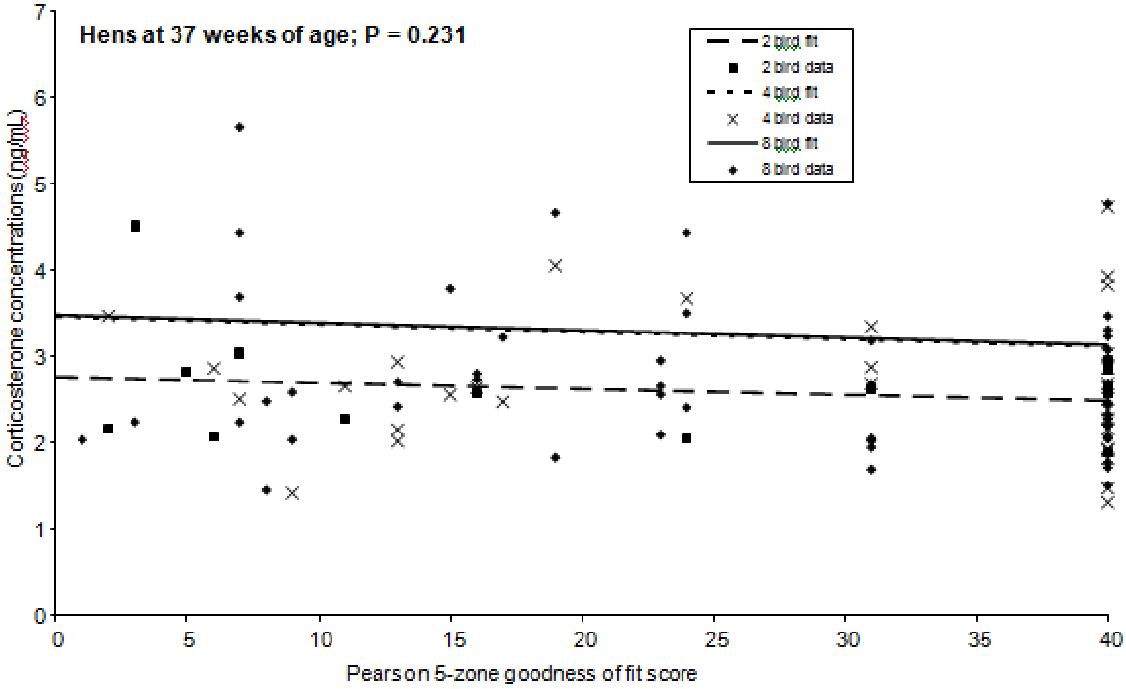
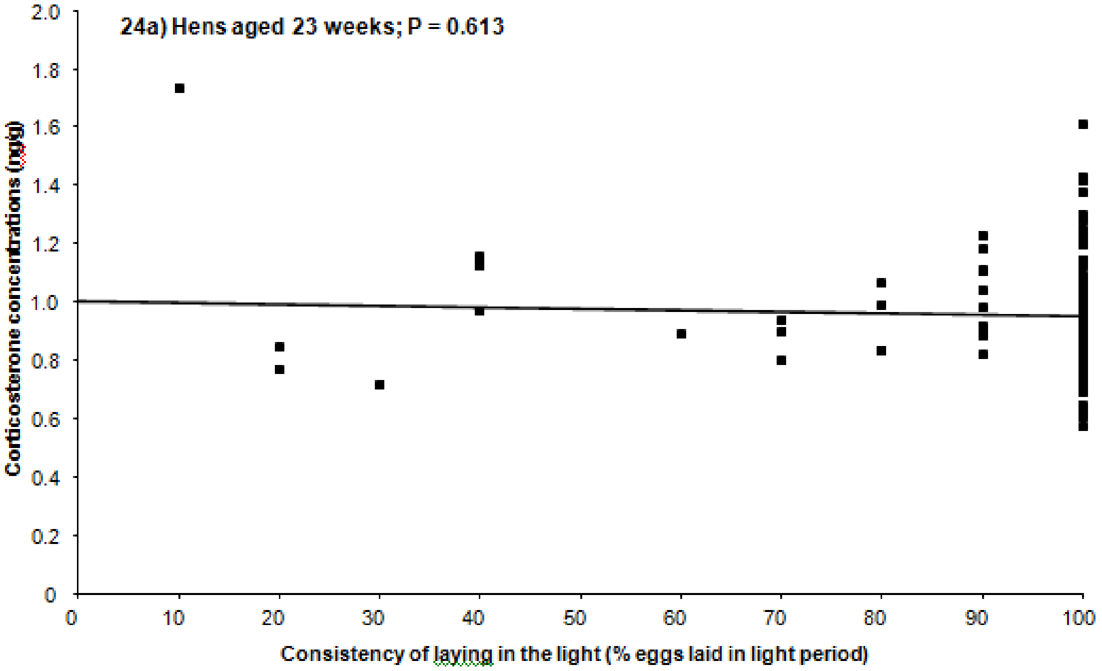
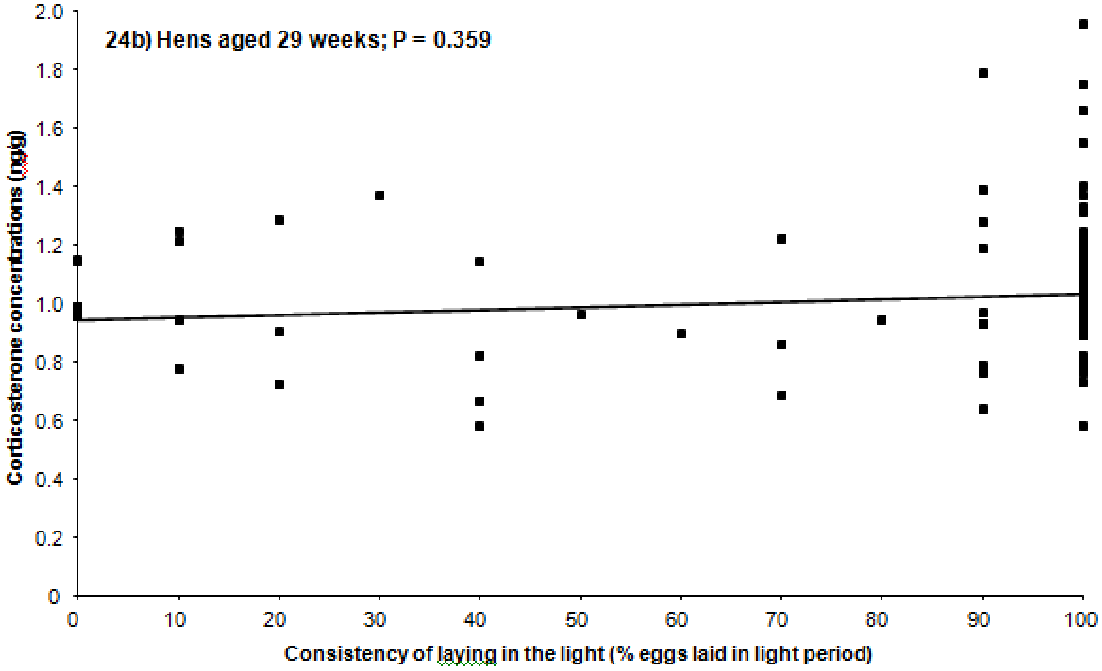


Figure 3-37 - Comparison of birds at 37 weeks of age in groups of 2, 4 and 8. The relationship between consistency of egg-laying site, based on a 5-zone Pearson's goodness of fit score and plasma corticosterone concentrations (ng/mL) adjusted for replicate, room and cage on the logarithmic scale. The 2 upper lines represent the means for hens in 4- and 8-bird cages and the lower line represents the mean for hens in 2-bird cages. The different symbols represent data points for individual hens from cages with different group sizes.

3.4.6.4 Consistency of egg laying in the light (and dark) and stress physiology measures

Hens were assessed at each of the 3 data sampling periods on the consistency of egg laying in the light, based on the previous 10 recorded eggs. Consistency was expressed as the percentage of eggs laid in the light. Egg albumen corticosterone concentrations were chosen as the measure of stress response for this comparison, as it was assumed there would be a closer relationship between the timing of the fusion of corticosterone into the albumen and oviposition than plasma corticosterone sampled at a fixed point in time in the early afternoon. The relationships between consistency of laying in the light and corticosterone concentrations in egg albumen were thus determined using REML analysis and are presented in Figure 3-38 for birds at 23, 29 and 37 weeks of age. As indicated in the series of figures, there were no significant relationships between consistency of laying in the light (or dark) and egg albumen corticosterone. Similarly, there were no significant relationships between consistency of egg-laying in the light and plasma corticosterone concentrations or H:L ratio.





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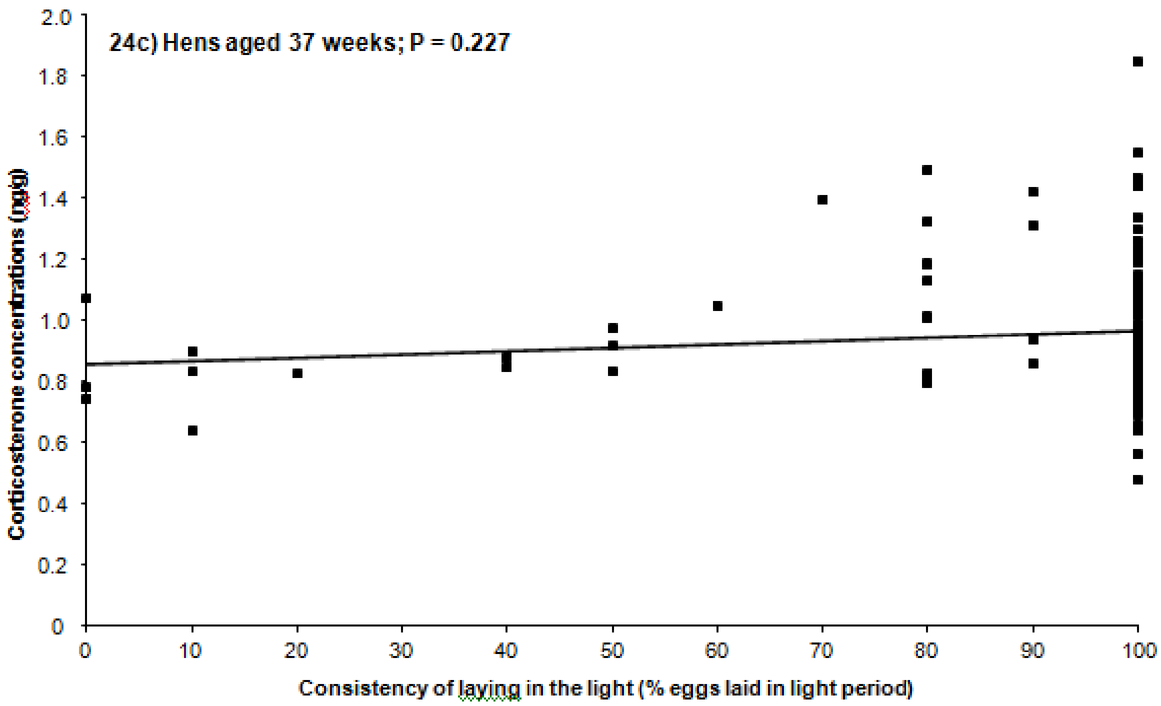


Figure 3-38 - The relationships between consistency of laying in the light and corticosterone concentrations in egg albumen (ng/g) at 23, 29 and 37 weeks of age. Corticosterone concentrations were adjusted for replicate, room and cage and analysed after logarithmic transformation.

3.5 Discussion

3.5.1 Nest box use and consistency of nest box use

Fewer than half of the 56 hens with access to a nest box laid their first egg in the nest box. Subsequently, the proportion of eggs laid in nest boxes increased linearly to about 70% at the 7th egg. However, from the 8h to the fortieth eggs, the proportion of nest box eggs remained relatively static. The finding thus suggests the use of the nest box for egg laying was reasonably consistent from the 8h egg onwards. Appleby and Smith (1991) found that although most eggs were initially not laid in nest boxes, the proportion of nest box eggs increased with time to between 70 and 90%, depending on nest box design. In the present experiment, while group size did not have a significant effect on the proportion of nest box eggs, nest box use appeared to be lower in 2-bird than 4- and 8-bird cages (see Figure 2-3). This result may be associated with the small number of cages involved (4 2-bird cages with a nest box), but is contrary to expectation. One expectation was that as the number of birds per cage (and per nest box) increased, then the proportion of nest box eggs would decline. Appleby (1984) suggested the maximum number of birds per nest box should not exceed 5 birds, although he indicated that in practice there were reports of up to 8 birds per nest box without floor egg problems. 2 relevant aspects of egg-laying behaviour and number of birds per nest box are (1) the physical size of the nest box and (2) the synchrony of egg laying by hens within a cage. Anecdotally, we regularly observed 5 birds in an 8-bird cage (cage 9, replicate 2) simultaneously in the nest box around the time of peak lay for the day, and generally all 8 hens laid in the nest box of this cage. While this was the extreme situation, it was not unusual to observe 3 birds simultaneously in a nest box.

The proportion of nest box eggs is a relatively coarse method for assessing consistency of nest box use, since no information is provided about individual birds. However, visualising the data set using histograms suggested a similar finding. Thus, by about the tenth egg laid, hens in cages with a nest box were consistent in their use of the nest box for egg laying. If consistency of laying was defined as ‘100% eggs laid in the nest box’, then the proportion of hens that laid all eggs in the nest box was 27%, 50%, 59% and 55%, respectively, per 10-egg cohorts over the first 40 eggs. In comparison there were 14%, 18%, 21% and 20% of hens, respectively, that laid nil eggs in the nest box in the 10-egg cohorts. If the definition of consistency was less strict, for example 80% or more eggs in the nest box, then 55%, 66%, 68% and 66% of hens, respectively, laid 8 to 10 eggs in the nest box across the cohorts. The proportion of hens that laid 0 to 2 eggs in the nest box per 10-egg cohort was 27%, 29%, 25% and 25% of hens, respectively. Of the remaining hens (inconsistent nest box layers that laid 3 to 7 eggs in the nest box), the proportion of hens per cohort of 10 eggs was 18%, 5%, 7% and 9%, respectively.

Thus, only about 2-thirds of hens were found to be consistent nest box users. The finding is lower than the recent reports by Tauson and Holm (2002, 2005), who surveyed Swedish poultry farms with furnished cages. Some farms, which would have had the same nest box as used in our experiment, had almost 100% nest box eggs. One factor contributing to the difference between our results and the Swedish surveys may have been the stain of birds. The birds in the Swedish surveys were white strains, whereas the birds in our experiment were brown (Hy-Line Brown). Appleby et al. (1983/84) found that level of light in the nest box influenced the initial use of the nest boxes by white but not brown laying strains. White pullets exclusively chose dark (5 lux) compared to light (40 lux) nest boxes for their initial egg. In contrast, only 29% of brown pullets chose dark nest boxes with the majority (71%) laying their first egg in the relatively bright 40 lux nest boxes. While it is well-known that light influences reproductive capability and behaviour in hens (Perry 2004), it is feasible that the reduced use of nest boxes for egg laying by the brown birds in our experiment was at least in part attributable to the birds’ breed/colour.

As expected, manipulating the light-dark schedule from 24 weeks of age by introducing a 3-h period of light from midnight in 1 of the 2 rooms, changed the timing of egg laying. Fewer, although not significantly fewer, eggs were subsequently laid in the nest boxes through to the completion of the experiment at 38 weeks of age, compared to the standard light schedule treatment. Birds are inactive in the dark (Tanaka and Hurnik 1991; Khalil et al. 2004) and as previously recorded by Sherwin and Nicol (1993a), the incidence of nest box eggs decreased if hens laid during the night period.

3.5.2 Consistency of oviposition site

2 relevant questions in this investigation on the consistency of egg-laying site were: (1) were nest box layers more ‘consistent’ than floor layers, and (2) how soon did hens become ‘consistent’ in their choice of egg-laying site, especially if no nest box was provided? In the present experiment, consistency of egg-laying site was estimated using the Pearson’s goodness of fit statistic, based on cage averages and a 5-zone distribution to enable comparison between cages with and without a nest box. While there were large statistical differences in the Pearson’s goodness of fit scores between cages with and without a nest box, the mean scores for both treatments, and thus the goodness of fit, indicated that on average birds were highly consistent in oviposition site, even during the first 10 eggs laid (see Table 3-10). The nest box, when provided, was clearly the preferred egg-laying site for the majority of birds. The statistical significance found between the nest box and no nest box treatments was perhaps not unexpected since the nest box was situated in a single location within cages (on the right side), which focused oviposition to 1

site in the cage, whereas in cages without a nest box, the majority of hens (52%) preferred to use 1 of 2 zones, situated at either side of the cage.

The time taken before hens could be considered ‘consistent’ in their egg-laying site in cages without a nest box, can be estimated from the change in mean Pearson’s goodness of fit scores over time (see Figures 3-10 and 3-11). The data suggest that floor layers were relatively consistent in their choice of egg-laying site between the twentieth and thirtieth eggs. While birds in cages without a nest box were slower to develop consistency for a particular site than nest box layers, as discussed above, this was probably not unexpected. Nevertheless, having a nest box in the cage appeared to hasten the consistency of oviposition site (in the nest box) by about 2 to 3 weeks. The significance of this for the welfare of laying hens is unclear.

3.5.3 Time and synchrony of oviposition

The start of egg laying each day, that is the average time the first egg per cage was laid each day, coincided with the time of lights-on, and the majority of eggs were laid within a relatively short time period. Highly synchronised egg laying is thought to create problems for birds in situations where there are insufficient nest boxes (Appleby 1984). In the smaller versions of furnished cages, which may accommodate up to 8 birds, only 1 nest box is provided. Timid or subordinate birds have been observed to enter the nest box to avoid bullying (Rudkin and Stewart 2003; Shinmura et al. 2006b, 2007). However, when egg-laying activity is highly synchronised within a cage, subordinate birds may avoid entering the nest box to avoid aggression. It is feasible that the combination of these factors may contribute to the incidence of floor eggs. In the present experiment the presence of a nest box in the cage resulted in increased synchrony of egg-laying times, particularly in the second and third quartiles when 50% of the eggs were laid. Modifying the light schedule from 23 weeks of age reduced the synchrony of egg-laying times and tended to reduce the incidence of nest box eggs, presumably reducing the demand by hens for use of the nest box.

3.5.4 Egg laying in the dark

By 23 weeks of age about half of the hens had laid at least 1 egg in the dark (see Figures 3-15, 3-16, 3-17 and 3-18). Based on the observations of Sherwin and Nicol (1993a) and Appleby and Hughes (1995), this was not unexpected during early lay. However, after 23 weeks, under the standard light schedule of 16 h continuous light to 8 h continuous dark, only 6 hens (10.7%) were recorded to lay in the dark. In contrast, during the same time period in the modified light schedule treatment, 77% of hens laid some eggs in the dark. Thus, the introduction of a 3 h period of light during the night had a major effect on the timing of egg laying.

As stated previously, 1 consequence of modifying the light schedule was an increase in eggs laid during darkness. The majority of birds that laid in the dark did not perform the typical pre-laying activity of birds that lay in the light. In general, oviposition occurred where the bird was located at the time. However, 8 birds continued to lay in the nest box (out of the total of 28 birds with access to a nest box in the Modified light schedule treatment). These birds did not perform ‘typical’ pre-laying activity (e.g. Appleby 1990; Duncan 1980). Rather, the birds walked into the nest box in the dark, laid their egg, then returned to the cage area. While this unusual behaviour does not appear to have been specifically reported in other studies of egg laying behaviour, Sherwin and Nicol (1993a) also reported that birds laid in nest boxes in the dark. Further, Appleby and Hughes (1995) reported a solution to floor eggs at a commercial farm where birds were laying in the dark before lights-on. The problem of floor eggs was resolved by opening the nest boxes 3 h before the lights came on in the morning. The implication was that hens walked to the nest boxes in the dark. In the present experiment, 3 of the birds that were consistent nest box layers in the dark were from 4-bird cages and 5 birds were from 8-bird cages.

At 23 weeks of age in the present experiment, there was an effect of group size on the consistency of hens laying in the dark. Proportionally more eggs were laid in the dark in 4-bird than 2- and 8-bird cages. This is an unexpected result and may be an anomaly related to social difficulties within certain 4-bird cages. Following the 23-week data collection point, the Modified light schedule commenced for one-half of the birds, and there was a general increase in the proportion of eggs laid in the dark in the Modified light schedule treatment. However, there were only weak ($P<0.1$) differences due to the light schedule main effect on the proportion of eggs laid in the dark around the 29 and 35 week data sampling periods.

3.5.5 Physiological stress responses to a nest box in the cage

Dawkins et al. (2004) concluded in relation to assessing hen welfare when different ‘indicators’ of welfare were combined, that measurements should occur at more than 1 point in time to take into account how the particular indicators may change with time. In the present experiment 4 physiological indicators were included: (1) plasma corticosterone from spot samples taken in the early afternoon, (2) egg albumen corticosterone, (3) blood haematology and (4) plasma corticosterone response to ACTH injection. Indicators #1 and #3 were measured on 3 occasions for each bird. For indicator #2, the first 20 eggs from each hen were identified so that corticosterone in the egg could be measured throughout the first few weeks of lay, as well as at each of the 3 data sampling periods. 4thly, plasma corticosterone concentrations were measured in response to an ACTH injection at the end of the experiment. Through such a combination of indicators it should be possible to detect physiological stress responses in hens, both direct endocrine responses of the HPA axis and the indirect effects of the endocrine responses on other systems such as the immune system, differentiate between acute (short-term) and chronic (long-term) stress responses and identify the environmental factors contributing to the stress response(s). There are many reports in the literature which demonstrate the endocrine effects of stressors on the HPA axis. For example, corticosterone concentrations have been measured in plasma, faeces and egg albumen in response to various stressors such as high stocking density/space allowance (Cunningham et al. 1987; Barnett and Cronin 2005), fear of humans and h8s (Barnett and Hemsworth 1989; Barnett et al. 1994; Fraisse and Cockrem 2006), handling and restraint (Beuving 1980) and relocation to a new environment (Dawkins et al. 2004; Downing and Bryden 2005).

In the present experiment there was no evidence over the first 20 eggs, or at any of the 3 sampling periods, that having a nest box in the cage reduced stress, as measured by corticosterone concentrations or blood haematology. However, at 23 weeks of age the presence of a nest box resulted in 33% higher plasma corticosterone concentrations, suggesting birds in cages with a nest box were more stressed. Further, the statistical analysis showed no difference in corticosterone concentrations due to the group size main effect, indicating that any reduction in floor space associated with the nest box perceived by the birds was not a major source of stress to the birds in this experiment, where floor space per hen at maximum group size was 600 cm^2 per bird. While the result may be associated with some form of social competition between birds for a resource, presumably the nest box, it is contrary to the perception that hens will be stressed if they are unable to lay in a nest box. Further, competition amongst the hens for the nest box ‘resource’ may be increased if egg laying becomes more synchronised. The extent of the assumed competition for the nest box may also be relevant to determining whether hens are excluded from using the nest box and become floor layers.

Regression analysis was used to investigate the relationships between nest box use, that is the consistency of use of the nest box for egg laying, and stress physiology variables at different times during the experiment. While there were no relationships at 23 weeks of age between the consistency of nest box use and plasma corticosterone or blood haematology, there was a highly significant quadratic relationship with egg albumen corticosterone concentrations. One interpretation of this relationship is that birds that were highly consistent in their egg-laying site selection (either 100% floor or 100% nest box layers) had higher stress levels, whereas birds that were 'less consistent' (ie. not at either extreme) were also less stressed. Alternately, the birds that were 100% floor layers may have been exclusively subordinates. Cunningham et al. (1987) found that subordinate birds in cages at high stocking densities had higher plasma corticosterone concentrations. While this is an interesting finding, the relationship was not found at the time when the data suggest selection of egg-laying site had become consistent (based on egg corticosterone concentrations in the fifth and tenth eggs). At the time of the fifth and tenth eggs laid, however, not all birds had commenced laying and there would have been less demand for the 'preferred' egg-laying sites. Similarly, there were no relationships at 30 and 35 weeks of age, suggesting the quadratic result may either be an artefact or a short-lived phenomenon.

Plasma corticosterone release is regulated by a circadian rhythm (Etches 1979). Within the diurnal pattern of release, Etches (1979) reported that the maximum corticosterone release occurred during the dark period, about 14 h prior to ovulation. Beuving and Vonder (1977), Etches (1979) and Johnson and van Tienhoven (1981) have shown a smaller, but highly variable peak in plasma corticosterone concentrations about 1-2 h prior to oviposition, the period in which hens show increased activity and prelaying behaviour (Duncan 1980; Duncan and Kite 1989). Presumably, if hens are frustrated and thus stressed at this time, it should be possible to detect differences in corticosterone. Beuving (1980) measured plasma corticosterone concentrations around egg laying in a small number of cannulated hens. The hens were housed singly in 1,000 cm^2 wire cages with or without a wooden nest tray containing litter, and no differences were found in plasma corticosterone between birds in the 2 the treatments around egg laying. In the present experiment spot samples for corticosterone were taken during a 1-2 h period commencing at 13.00 h. According to Etches (1979), corticosterone in plasma should be at resting levels at this time. Neither the intervals from egg laying to blood sampling (calculated later from the video record), nor removal from the cage to blood sampling, were found to be related to plasma corticosterone concentrations. Only those plasma samples collected within 3 min of capture of the birds were included in the data analysis, as plasma corticosterone concentrations become elevated within minutes following handling and restraint of laying hens (Beuving 1980; Fraisse and Cockrem 2006).

The difference between the findings for plasma and egg albumen may be related to the 'sampling period'. Corticosterone transfers into albumen and accumulates during the period 3-5 h after ovulation (Downing and Bryden 2005) followed by a period of about 4 h of slow calcification when the shell membrane remains semi-permeable. Plasma was collected at a point of time, possibly coinciding with the 3-5 h period of albumen formation. The time of egg-laying for individual birds was relatively variable. In the context of this experiment therefore, we do not necessarily anticipate egg albumen corticosterone concentrations will be indicative of events affecting the hen in the pre-laying period (e.g. 2 h pre-oviposition). Depending on the timing of events, and these are by no means known with any certainty, egg albumen corticosterone concentrations may reflect the blood plasma concentrations during a period when the hen may have motivations other than those associated with egg-laying, perhaps feeding, resting, roosting or sleeping.

While there were no differences in plasma or egg corticosterone concentrations due to the group size main effect at the first and second blood sampling periods (23 and 30 weeks, respectively), there was a difference in plasma corticosterone at 37 weeks. 2-bird cages had lower (P=0.015) plasma corticosterone concentrations than cages with 4 or 8 birds. While this finding is consistent with the literature that increased group size (and reduced space per bird) elevate corticosterone concentrations in plasma (Cunningham et al. 1987), there were no differences detected in egg corticosterone. As suggested above, this may be indicative of short-term (acute) stress responses. In addition, there were no differences due to the main effects and there were no interactions in H:L ratio or maximum plasma corticosterone in response to ACTH injection. Thus, none of the stress-related measurements in the experiment were suggestive of any long-term change in HPA function and hence a chronic stress response.

An unusual finding of this experiment was that twice as many hens in cages without a nest box laid multiple eggs on at least 1 day compared to hens in cages with a nest box. Where the second egg was laid within a few h of the first egg, it was generally soft-shelled and thus could not be used in a commercial sense. The aetiology of laying multiple eggs per day is thought to be related to genetic selection for prolificacy in modern layer strains. How and why multiple egg-laying occurred less frequently in birds in cages with a nest box is not known, but it could be related to stress. Stress tends to adversely affect reproduction. In the present experiment there was evidence in the early stages of lay of higher stress levels in cages containing a nest box.

3.5.6 Conclusions

In conclusion, a major finding of this experiment was that the presence of a nest box in the cage increased the stress response of birds aged 23 weeks, as measured by plasma corticosterone concentrations. The higher stress was possibly associated with resource competition in early lay. The results of the experiment also suggest that hens which become consistent nest box layers, do so by about their tenth egg. Birds without a nest box seem to become consistent in their egg-laying site perhaps 2 weeks later. Birds that were highly consistent in their egg-laying site, that is always used the same site over their previous 10 eggs, were found to have higher egg corticosterone concentrations, possibly indicating that at that stage in egg-laying, they were less adaptable to change in their environment.

While the estimated 2-week difference in achieving consistency of egg-laying site between nest box and floor layers may in some way have contributed to the effect of nest boxes elevating plasma corticosterone at 23 weeks, overall, most hens consistently used the nest box and there were no long-term adverse effects on stress physiology between hens that laid in a nest box compared to hens that laid on the wire floor. Thus, whatever importance hens attach to a nest box, it is insufficient to result in long-term effects on the HPA axis.

Summary of findings:

- The presence of a nest box in the cage did not reduce the risks to bird welfare, based on stress physiology.
- Birds that were floor layers did not have poorer welfare than nest box layers, based on stress physiology.
- Increasing the ratio of birds per nest box did not reduce the proportion of nest-box eggs.
- Increasing the ratio of birds per nest box did not increase the risk to bird welfare based on stress physiology. Increasing the number of birds per cage did increase the risk to bird welfare at 37 weeks, based on plasma corticosterone concentrations.

- Manipulating the light to dark schedule to introduce a period of light during the ‘night’ altered the timing and synchrony of egg laying.
- Birds that laid in the dark did not have poorer welfare than birds that laid in the light. [A proportion of birds that consistently laid in the dark were noted to perform an unusual form of pre-laying behaviour in that they walked to the nest box, laid their egg and exited the nest box in the dark].

4 Experiment 2 - The effects of abrupt versus gradual introduction of light during the dark period on the synchrony of egg laying, the incidence of eggs laid in the dark and the use of the nest box.

4.1 Background

The welfare of laying hens housed in cages is a current international topic of ethical, political and scientific debate. In the European Union from 2013, the production of eggs from caged hens will only be acceptable if ‘furniture’ is provided in the cage. Furniture refers to a nest box, dust bath and perch. In the AECL and DPI co-funded project DAV197A - Welfare of laying hens in furnished cages (Barnett and Cronin 2005), the provision of a nest box did not improve the welfare of hens based on stress physiology, immunological competence, and feather and foot condition scores. About 2-thirds of eggs were laid in the nest box, which was similar to the 70% recorded in experiment 1 of the present project. Thus, in both experiments the majority of hens used the nest box for egg-laying. Nevertheless, about one-third of the birds did not use the nest box provided, with the majority of these hens choosing a consistent ‘nest site’ on the wire floor outside the nest box.

A key criticism of cages without nest boxes is that pre-laying behaviour is frustrated (Duncan 2001). The finding that most hens lay in a nest box when provided (Tauson and Holm 2002, 2005) is a major argument supporting the belief that nest boxes are important to hen welfare (Weeks and Nicol 2006). Keeling (2004) has summarised the views of previous authors in this respect: “if a hen is motivated to lay in a nest, but cannot find what to her is an appropriate site so, as a last resort, lays in an inappropriate place, then it probably is a welfare problem.” The birds in the experiment of Barnett and Cronin (2005) and experiment 1 of this project that consistently chose to lay outside the nest box presumably considered they were laying in an appropriate site for their requirements. In addition, based on the stress physiology measures recorded in the 2 experiments, there were no indications that bird welfare was adversely affected. The possible exception to this was a small number of birds in experiment 1 of the present project, housed in cages with a nest box, that either laid all eggs in the nest box (ie. 100% consistent) or all eggs outside the nest box (ie. 100% floor-layers). While both classes of birds showed evidence of elevated corticosterone concentrations in egg albumen at 23 weeks of age (compared to birds that were less consistent), relationships between consistency of egg-laying site and physiological stress measures were not found at other times.

From studies of the pre-laying activities of domestic hens, it is generally accepted there are 2 phases of behaviour involved in oviposition (Sherwin and Nicol 1992). Beginning 1 to 2 h prior to oviposition, the activity level of hens increases in a phase of behaviour termed 'searching' in which hens appear motivated to seek a nest site. In this phase hens increase locomotion and perform behaviours such as inspection of potential nests. The function of pre-laying activity in wild populations seems to be to facilitate hens locating an appropriate (secluded and secure) place for egg laying and subsequent incubation of the eggs (McBride et al. 1969; Duncan et al. 1978). Once hens have selected their nest site the 'sitting' phase commences, which includes the adoption of a sitting posture interspersed with nest-building activities such as scratching the floor/litter, rotating the body on the nest and collecting litter if available.

Activities performed in the searching phase are goal-directed or appetitive behaviours, occurring when hens are motivated to find a suitable nest for oviposition (the consummatory behaviour). Thus, Appleby and McRae (1986) and Duncan and Kite (1989) showed that hens were motivated to lay their egg in a nest box, and if a nest box was not available hens performed more nest-searching behaviour (Cooper and Appleby 1995; Freire et al. 1996). While an increased occurrence of appetitive behaviour may indicate a stronger motivation to achieve the consummatory phase, it does not necessarily indicate that increased pre-laying locomotion reflects increased frustration and thus a potential welfare problem. For example, using an aversive task approach, Freire et al. (1997) suggested that hens were only weakly motivated to reach the nest site during the searching phase, although the motivation to gain access to a nest site increased near the start of the sitting phase.

A potential reason why egg laying might occur outside the nest box is that, in the early stages of lay, hens occasionally lay before lights-on. The period of early lay, perhaps up to when the hen has laid her tenth egg, appears to be the time when the preference for egg-laying site develops. As recorded in experiment 1, about one-half of hens were recorded to lay at least 1 egg in the dark prior to 23 weeks of age (see Figures 3-15, 3-16. 3-17 and 3-18). Laying hens are inactive in the dark (Tanaka and Hurnik 1991; Khalil et al. 2004) and those that lay in the dark do not perform typical pre-laying activities (Sherwin and Nicol 1993a; Sharp 1993; Appleby and Hughes 1995), potentially decreasing the incidence of nest box eggs (Sherwin and Nicol 1993a). The question is therefore raised regarding the effect of light per se on the motivation of hens to perform pre-laying, nest-seeking activities. Experimental studies have shown that manipulating light-dark schedules, and in particular the time of actual lights-off and 'expected sunset' (viz. the time of day birds expect the lights to go off, which may be under the control of an internal time clock) effect the timing of LH release, ovulation and oviposition in laying hens (Morris 1973; Lewis et al. 2007a and b). Are hens that lay in the dark motivated to perform nest seeking activity? The results from experiment 1 showed no differences in the relationships between laying in the dark compared to light and physiological stress indicators, suggesting the welfare of hens that lay in the dark is not adversely affected. Thus, if hens lay in the dark their perception may be that they may have already reached the second phase of pre-laying behaviour, that is 'sitting on their preferred nest site', whether that be in a nest box or on the wire floor. Does this, however, mean that their welfare is better or worse?

The observation that hens which lay during darkness do not display the high activity 'searching' phase of pre-egg-laying behaviour provides a model for comparison of the importance of the 'searching' phase for hen welfare. Morris (1973) and others, including the results from experiment 1 of this project, demonstrated the possibility of shifting the timing of egg laying through manipulation of the light schedule so that egg laying occurred in darkness, outside the nest box. Nevertheless, some hens in experiment 1 did enter the nest box in the dark, laying their egg, then leaving the nest box. Experiment 1 also found that a proportion of hens did not respond to the light-dark schedule manipulation and continued to lay in the light period. Of these hens, some utilised the nest box for egg laying whereas others did not. A practical outcome of hens laying in darkness could be that fewer birds require access to the nest box thereby reducing the potential stress experienced by young birds trying to enter the nest box during times of peak lay in the cage. Further, egg laying in darkness should result in less concentration of eggs in front of the nest box or at the corners of ages on the egg collection tray, thus reducing the risk that eggs are cracked or broken.

4.2 Objectives and hypotheses

The main objective of this experiment was to investigate the effects of exposing young laying hens to a modified light-dark schedule involving the introduction of a light period during the night. The experiment sought to determine the effects of 2 methods of light introduction on egg-laying patterns and stress response of birds. The rationale of introducing a light period to hens during their night was to shift the time of egg laying such that, for a proportion of hens, oviposition occurs in the dark. Egg laying in the dark serves a number of other objectives. For example, the level of competition for the nest box would be reduced as most of the hens would lay outside the nest box. Experiment 1 found that birds in cages with a nest box experienced an acute stress response at 23 weeks of age due to the nest box. Further, egg-laying in darkness is not accompanied by the typical ‘active’ pre-laying behaviour by hens, which according to opponents of cage housing systems is a major sign of frustration and thus poor welfare in laying hens.

The 2 treatments in experiment 2 compared the manner in which the light period was introduced:

4.2.1 Gradual Light Introduction Treatment:

Commencing at 18 weeks of age, birds were exposed to 30 min of light during the night, ending at about 03.00 h. While maintaining the end of the inserted light period at about 03.00 h each day, the duration of the light period was increased by 30 min per week until 23 weeks of age, when the birds received 3 h of light (at night) within their overall photoperiod of 16 h light to 8 h dark.

4.2.2 Abrupt Light Introduction Treatment:

Commencing at 23 weeks of age, birds were exposed to 3 h light, inserted from midnight to 03.00 h within their photoperiod of 16 h light to 8 h dark.

4.2.2.1 Hypotheses

- Manipulation of the light-dark schedule and abrupt versus gradual introduction of a period of light during the night do not reduce bird welfare based on stress physiology.
- Manipulation of the light-dark schedule and the introduction of a period of light during the night alters the peak time of oviposition, coinciding with the dark period.
- Manipulation of the light-dark schedule and the introduction of a period of light during the night results in consistently fewer eggs laid in nest boxes.

4.3 Materials and methods

The experiment was conducted in the same 2 controlled environment rooms used in experiment 1 and involved 96 Hy-Line Brown hens. The hens were produced from hatching eggs purchased from a commercial hatchery and reared from day old to 13 weeks in group cages in a controlled environment shed. Chicks were vaccinated at day old with Rispens Mareks vaccine (Bioproperties Australia) and at 12 weeks of age with killed Newcastle Disease vaccine (Nobilis, Newcavac, Intervet). Pullets were not beak trimmed and they were reared using conventional commercial lighting programs and nutrition as supplied by Ridley Agri-products. At 13 weeks the birds were transported to DPI Werribee and placed in cages in groups of 8. There were 6 experimental cages per room and 2 rooms. All cages contained a nest box, although the entrances to the nest boxes were closed until the birds were 15 weeks. Light manipulation treatments were allocated to rooms and the same photoperiod (ie. total number of h of light per day), which increased from 12 h at 13 weeks to 16 h at 23 weeks (Figure 4-1), was applied to both rooms.

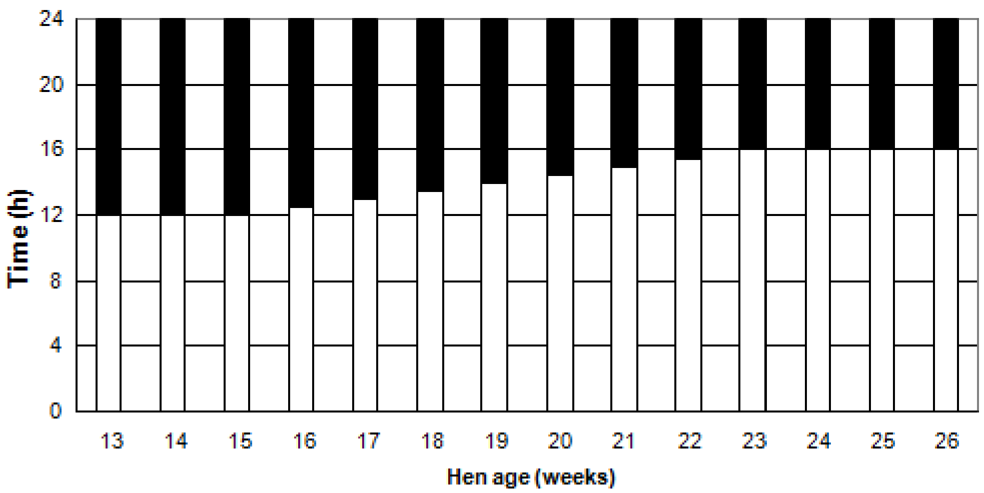


Figure 4-1- The change in total amount of light (open column sections) and dark (solid column sections) per day imposed on birds over the course of the experiment.

The experiment involved exposing birds to a period of light during the night, while the photoperiod was also being increased. The light period was inserted either gradually or abruptly (Table 4-1; Figure 4-2). Treatments were assigned at random to the 2 rooms available, and lighting in the 2 rooms was independently controlled. Thus, treatments were unavoidably confounded with room.

Table 4-1- Light to dark ratio schedules used in Experiment 2 in the different treatments.

Hen age (wks)Gradual light introduction treatment (h)	Light (L) to Dark (D) Abrupt light introduction treatment (h)	Light (L) to Dark (D)
13 to 15	12 L :12 D	12 L :12 D
16	12.5 L : 11.5 D	12.5 L : 11.5 D
17	13.0 L : 11.0 D	13.0 L : 11.0 D
18	13.0 L : 7.5 D : 0.5 L : 3.0 D	13.5 L : 10.5 D
19	13.0 L : 7.0 D : 1.0 L : 3.0 D	14.0 L : 10.0 D
20	13.0 L : 6.5 D : 1.5 L : 3.0 D	14.5 L : 9.5 D
21	13.0 L : 6.0 D : 2.0 L : 3.0 D	15.0 L : 9.0 D
22	13.0 L : 5.5 D : 2.5 L : 3.0 D	15.5 L : 8.5 D
23 to 26	13.0 L : 5.0 D : 3.0 L : 3.0 D	13.0 L : 5.0 D : 3.0 L : 3.0 D

Birds were placed in the cages at 13 weeks of age.

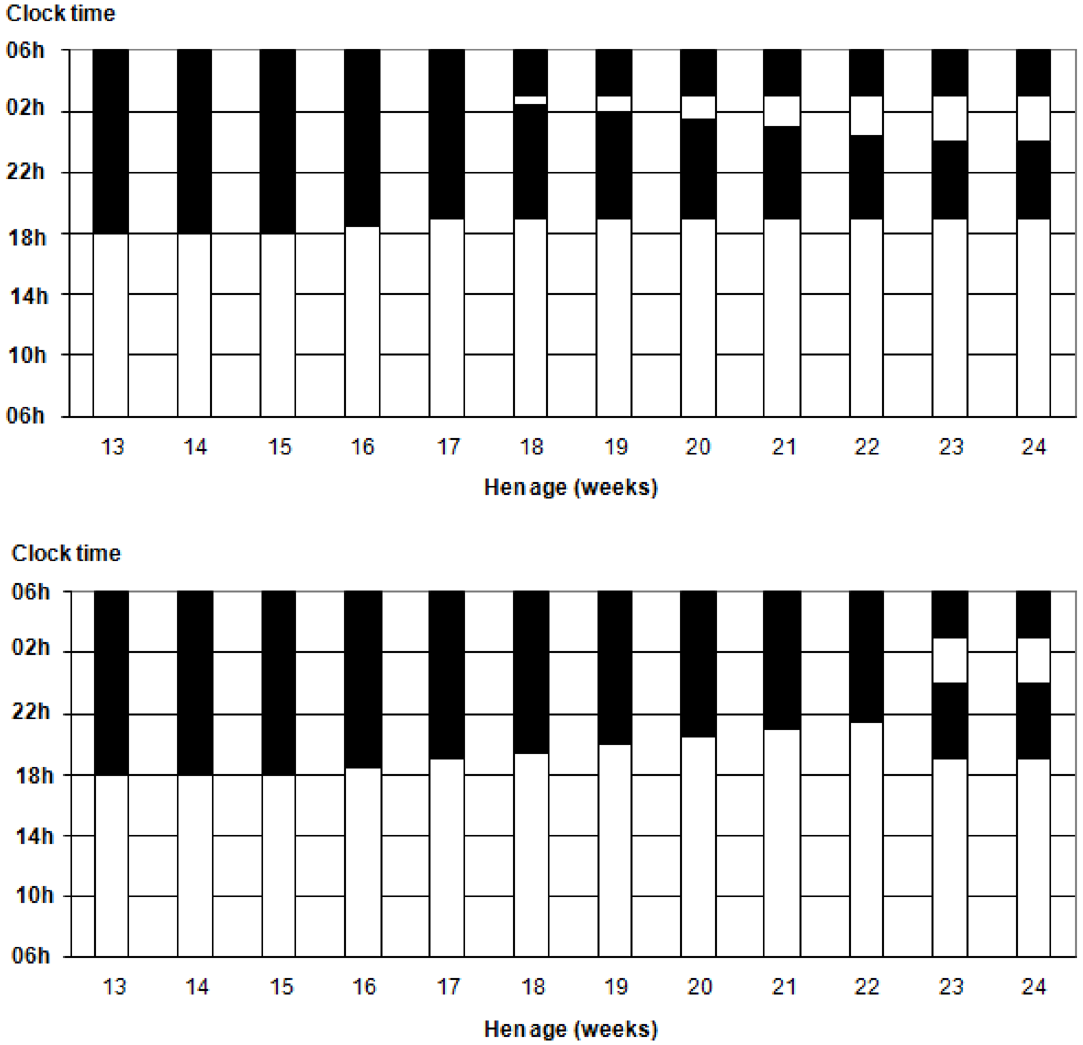


Figure 4-2- Description of how the light and dark intervals changed through the experiment in the Gradual (upper graph) and Abrupt Light Introduction (lower graph) treatments. Light and dark periods are denoted by open and solid column sections, respectively. Birds in the 2 treatments received the same total amount of light per day.

Low-light cameras were positioned below each cage and inside each nest box to provide a view of the 96 birds at all times and enable the time of egg laying to be recorded. A combination of black and white leg bands was used, as in experiment 1, to enable birds and the time and location eggs they laid to be identified from the digital video record. Using this technique it was also possible to identify the specific eggs laid by each hen at a later date from the video record, for measurement of egg albumen corticosterone concentrations.

On days when eggs were to be sampled, the experimenter first made a grid map record to show the approximate location of all eggs in each cage, viz. in the nest box, on the roll-out tray or inside the cage. The grid map also identified the cage number, date, time of collection and initials of person recording the information and a sequence number was allocated to each egg marked on the grid. The sequence number was also written in pencil on the egg (shell). Eggs were then collected and placed on trays. Once all eggs in the room had been collected, the trays of eggs were taken to the laboratory where each egg was weighed whole before being broken to separate the albumen from the yolk. The albumen was weighed then frozen for later analysis of corticosterone concentrations. The video records were examined to match eggs to the hens that laid them. The frozen albumen was stored until transported to Camden, NSW, where it remained frozen until analysed for corticosterone concentrations using the method developed by Downing and Bryden (2005). Total corticosterone concentrations in plasma were assayed using a commercial diagnostic kit (ICN ImmuChem Double antibody RIA, 7 Hills, NSW) under the supervision of Dr Jeff Downing, Faculty of Veterinary Science, University of Sydney.

Corticosterone transfers into albumen and accumulates during the period 3-5 h after ovulation (Downing and Bryden 2005) followed by a period of about 4 h of slow calcification when the shell membrane remains semi-permeable. The time between closing of exchange of corticosterone from the blood supply to the albumen occurs approximately 12 h (±6 h) prior to egg laying, although is not known with any certainty. In the context of this experiment therefore, we do not necessarily anticipate egg albumen corticosterone concentrations will be indicative of events affecting the hen in the period she performs pre-laying behaviour (e.g. 2 h pre-oviposition). Assuming oviposition occurs in the morning, the egg albumen corticosterone concentrations should reflect the blood plasma concentrations during the period of late afternoon and evening (night) when the hen will have different motivations, perhaps resting, roosting and sleeping.

4.3.1 Statistical analysis

Analysis of variance was used to examine differences due to the light introduction treatments (GenStat 10.1, Lawes Agricultural Trust) on egg-laying characteristics and egg corticosterone concentrations. The experimental unit was the cage of birds.

4.4 Results

4.4.1 Egg production

There was no difference between the 2 light schedule treatments in egg production during the measurement period in the experiment (Figure 4-3).

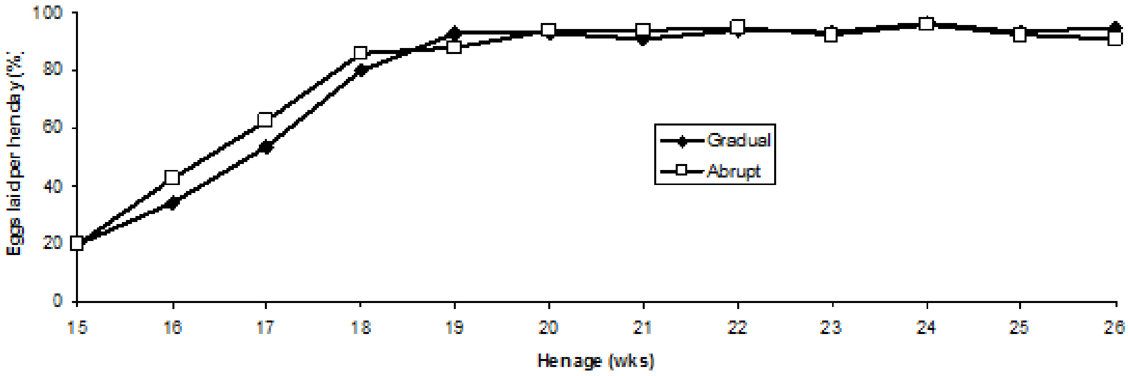


Figure 4-3 - Egg production in the 2 light manipulation treatments. Introduction of light during the night time commenced at 18 and 23 wks, respectively, for the Gradual and Abrupt Light Introduction treatments. From 23 wks both treatments received 3 h of light from midnight.

4.4.2 Timing and synchrony of egg laying

The timing of egg laying by hens in the 2 treatments over 12 weeks of video observation is shown in Appendix I. The data are presented as the frequency of eggs laid per hour. The effects of treatment on the timing of egg laying were determined by analysis of the median time of egg laying (Table 4-2). As shown in Table 4-2, between 18 and 23 weeks of age, the median egg laying time for birds in the Gradual Light Introduction treatment was about 1.5 h earlier each day than hens in the Abrupt Light Introduction treatment. After 23 weeks, when both treatments received the same quantity of introduced light during the night, the median times were not different.

Table 4-2 - Median time of egg laying by hens. Values shown are cage mean clock times (h). The numbers in parentheses are the mean number of eggs recorded per cage over the 4 days of video observation.

Hen age (wks)	Light Introduction Treatment				
	Gradual	Abrupt	SED	P Value	Event / Comments
15	8.83 (6.3)	9.08 (6.3)	0.958	0.80	
16	9.25 (11.0)	7.67 (13.2)	0.625	0.030	
17	7.67 (17.2)	7.67 (20.0)	0.553	1.00	
18	6.33 (24.5)	7.50 (26.3)	0.601	0.081	Start of Gradual treatment
19	5.75 (30.0)	7.67 (24.7)	0.473	0.002	
20	6.50 (28.5)	7.50 (30.2)	0.316	0.010	
21	5.67 (29.3)	7.33 (30.2)	0.298	<0.001	
22	6.83 (26.5)	8.50 (21.5)	0.511	0.009	
23	6.33 (28.5)	8.08 (28.2)	0.620	0.018	Start of Abrupt treatment
24	6.83 (30.8)	7.42 (30.7)	0.659	0.397	
25	7.33 (30.0)	7.25 (30.0)	0.554	0.883	
26	7.50 (29.2)	7.58 (29.3)	0.625	0.897	

SED: standard error of difference between the means

While light introduction changed the median egg laying time, there were no differences over the course of the experiment in the synchrony of egg laying, as estimated by the time taken for the middle 50% of eggs to be laid per cage (ie. the second and third quartiles combined; Table 4-3). Increased synchrony of egg laying will be represented by lower values and less-synchronised egg laying by higher values.

Table 4-3 - Synchrony of egg laying, estimated by the time taken for the ‘middle 50%’ of eggs to be laid, ie. the second and third quartile of eggs. Values shown are cage mean clock times (h).

Hen age (wks)	Light Introduction Treatment				
	Gradual	Abrupt	SED	P Value	Events/ comments
15	2.9	2.9	0.61	1.00	
16	2.9	2.8	0.80	0.84	
17	2.4	2.2	0.64	0.80	
18	2.5	2.2	0.62	0.60	Start of Gradual treatment
19	2.3	1.7	0.36	0.14	
20	1.7	2.2	0.52	0.36	
21	2.0	2.0	0.45	1.00	
22	2.0	1.5	0.59	0.42	
23	2.8	2.3	0.73	0.51	Start of Abrupt treatment
24	2.3	2.2	0.85	0.89	
25	2.8	2.7	0.53	0.82	
26	2.5	3.0	0.81	0.55	

SED: standard error of difference between the means

4.4.3 Egg laying in the dark

Following the introduction of as little as 30 min of light during the ‘normal’ night time in the Gradual Light Introduction treatment, about 50% of eggs were recorded to be laid during darkness (Figure 4-4 and Table 4-4). While increasing the duration of introduced light did not increase the proportion of eggs laid in the dark in the Gradual treatment, the apparent reduction in eggs laid in the dark over time suggests the birds may have become photo-refractory by about 25 weeks of age. As shown in Figure 4-4, the proportion of eggs laid in the dark in the Abrupt treatment increased about 20-30% to about 50% after introduction of the light period at 23 weeks.

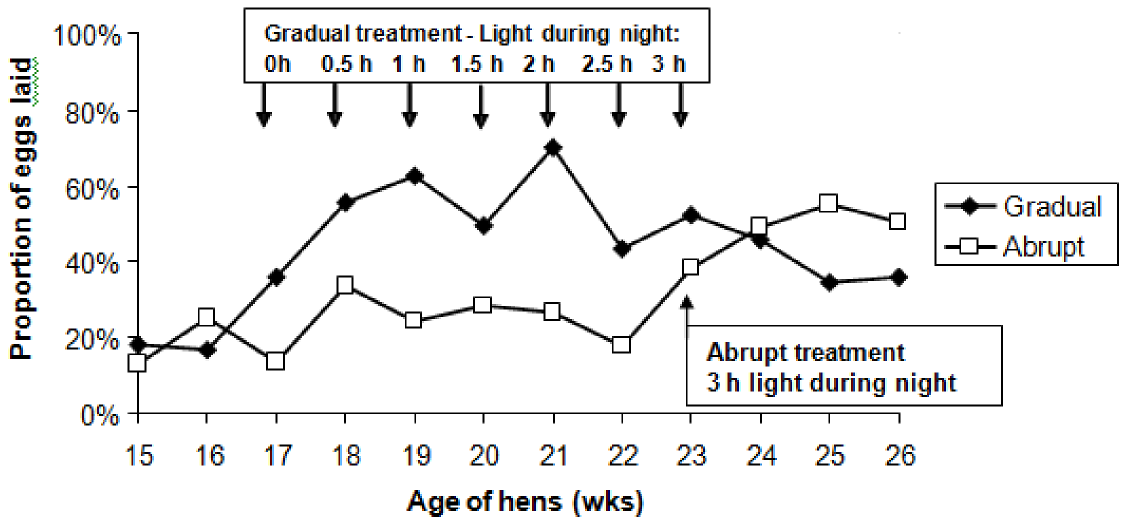


Figure 4-4 - Eggs laid in the dark by birds in the Gradual and Abrupt Light Introduction treatments. Both treatments received the same total amount of light per day (see Table 3-1). The values shown are cage averages.

Table 4-4 - Egg laying in the dark. The proportion of eggs laid during darkness by birds in the 2 light introduction treatments. Values shown are cage mean percentages.

Hen age (wks)	Light Introduction Treatment				
	Gradual	Abrupt	SED	P Value	Event
15	19.7	17.8	12.96	0.89	
16	16.7	26.8	8.98	0.29	
17	33.7	14.2	8.58	0.047	
18	56.3	35.0	11.40	0.091	Start of Gradual treatment
19	62.4	25.6	8.60	0.002	
20	49.8	28.7	10.60	0.075	
21	69.7	26.6	9.57	0.001	
22	42.6	17.1	8.25	0.011	
23	53.1	37.8	11.80	0.22	Start of Abrupt treatment
24	45.9	48.6	10.26	0.80	
25	33.8	54.7	9.11	0.044	
26	35.5	50.2	10.40	0.19	

SED: standard error of difference between the means

As expected, the majority of eggs laid in the dark were laid between 03.00-06.00 h (Figure 4-5 and Table 4-5).

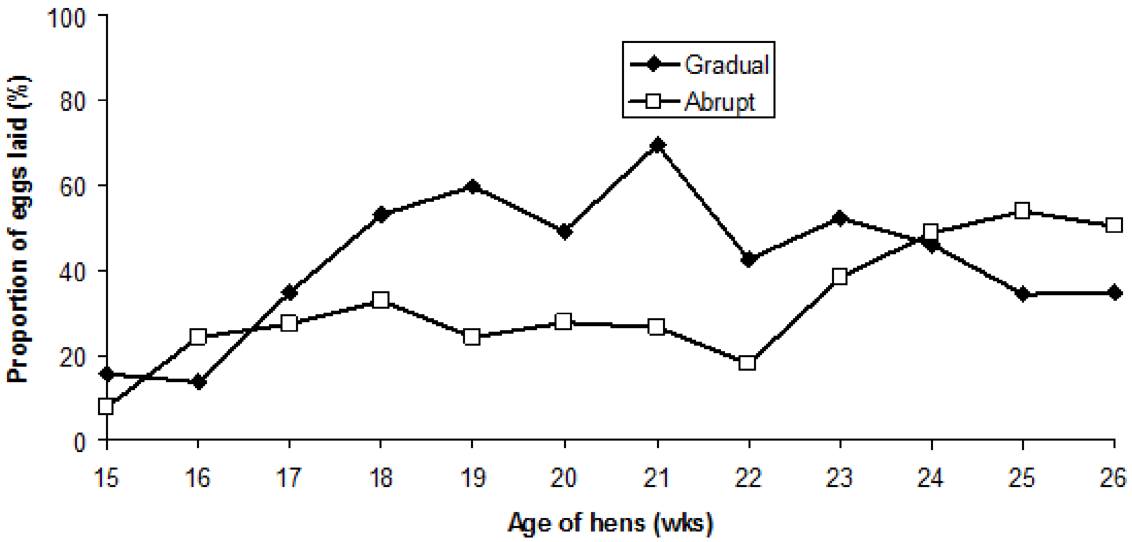


Figure 4-5- Eggs laid in the dark between 03.00 and 06.00 h by birds in the Gradual and Abrupt Light Introduction treatments. Both treatments received the same total amount of light per day (see Table 4-1). The values shown are cage averages.

Table 4-5 - Egg laying between 03.00 and 06.00 h (in the dark) by birds in the 2 light introduction treatments. Values shown are cage mean percentages.

Hen age (wks)	Light Introduction Treatment				
	Gradual	Abrupt	SED	P Value	Event
15	16.9	14.4	13.24	0.21	
16	13.6	25.6	8.15	0.17	
17	32.9	12.4	8.21	0.031	
18	53.8	34.4	11.96	0.14	Start of Gradual treatment
19	59.5	25.6	8.74	0.003	
20	49.2	27.6	10.85	0.074	
21	69.2	26.6	9.42	0.001	
22	40.0	17.1	8.62	0.024	
23	53.1	37.8	11.80	0.22	Start of Abrupt treatment
24	45.9	48.6	10.26	0.80	
25	33.8	53.7	8.77	0.047	
26	34.4	50.2	10.34	0.16	

SED: standard error of difference between the means

4.4.4 Egg laying in the nest box

Birds in the Gradual Light Introduction treatment laid about 70% of their eggs in the nest box. In comparison, the proportion of nest box eggs was lower (about 50%) for the Abrupt Light Introduction treatment (Figure 4-6 and Table 4-6).

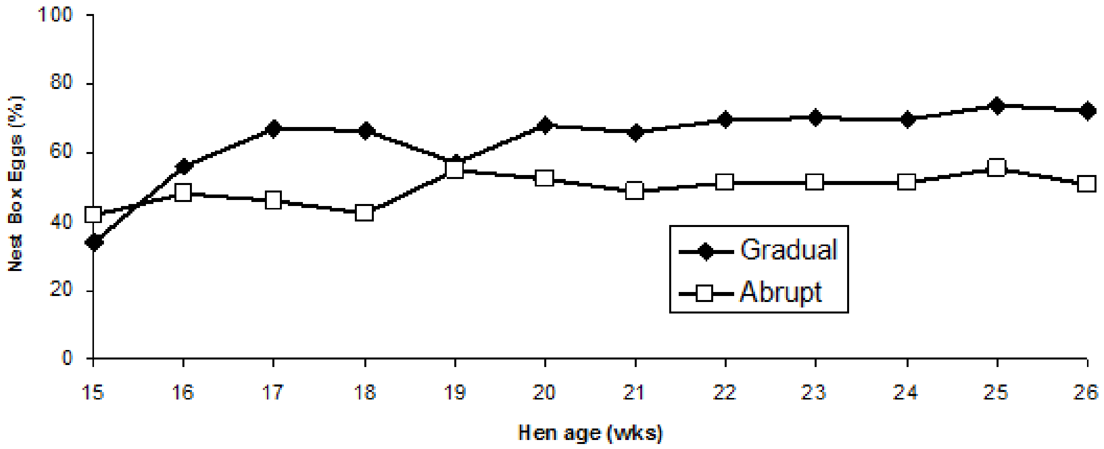


Figure 4-6- Change in the proportion of eggs laid in the nest box over time, in the Gradual and Abrupt Light Introduction treatments. Values shown are cage averages. The light introduction commenced at 18 weeks for the Gradual treatment and 23 weeks for the Abrupt treatment.

Table 4-6 - The proportion of eggs laid in the nest box per week by birds in the 2 light introduction treatments. Values shown are cage mean percentages.

Hen age (wks)	Light Introduction Treatment				
	Gradual	Abrupt	SED	P Value	Event
15	32.1	39.4	16.45	0.67	
16	57.0	47.8	16.71	0.59	
17	68.6	46.3	13.43	0.13	
18	66.8	42.1	10.95	0.048	Start of Gradual treatment
19	57.2	51.9	12.24	0.68	
20	67.0	52.7	10.29	0.20	
21	66.2	48.8	11.09	0.15	
22	65.9	51.7	11.08	0.23	
23	70.2	50.5	11.01	0.10	Start of Abrupt treatment
24	69.6	51.3	8.19	0.049	
25	74.2	55.8	9.43	0.079	
26	72.5	50.9	10.32	0.063	

SED: standard error of difference between the means

Egg laying in the nest box between 03.00 and 06.00 h

In both treatments the introduction of light during the 'normal' night period resulted in an increased proportion of eggs laid in the nest box between 03.00 and 06.00 h, that is during darkness (Figure 4-7 and Table 4-7). It is assumed that by 18 weeks of age, the latter birds in the Gradual Light Introduction treatment were already consistent nest box layers, with an established pattern of laying in the nest box. Similarly, for the Abrupt Light Introduction treatment from 23 weeks of age, birds that laid in the nest box in the dark were probably consistent nest box layers with a high motivation to lay in the nest box.

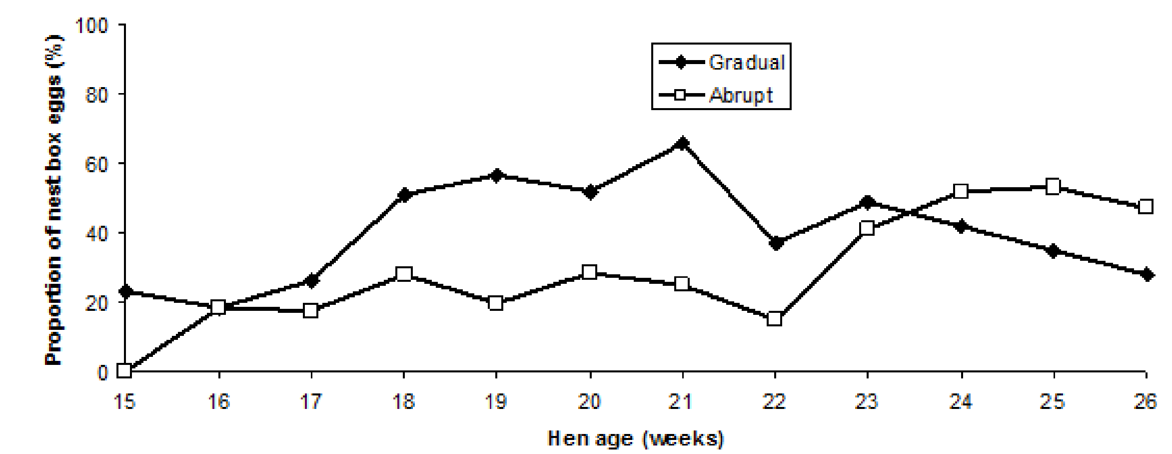


Figure 4-7- The proportion of nest box eggs laid between 03.00 and 06.00 h (ie. in the dark) in the Gradual and Abrupt Light Introduction treatments. Light introduction commenced at 18 weeks for the Gradual treatment and 23 weeks for the Abrupt treatment. Values shown are based on cage averages.

Table 4-7 - The proportion of eggs laid in the nest box between 03.00 and 06.00 h per week by birds in the 2 light introduction treatments. Values shown are cage mean percentages.

Hen age (wks)	Light Introduction Treatment				
	Gradual	Abrupt	SED	P Value	Event / Comment
15	22.9	0.0	-	-	Insufficient data for analysis
16	18.1	18.4	12.65	0.98	
17	26.2	17.4	13.06	0.52	
18	51.0	27.7	15.46	0.16	Start of Gradual treatment
19	56.5	19.4	15.18	0.035	
20	51.8	28.2	14.37	0.13	
21	65.8	24.8	12.45	0.008	
22	36.8	14.6	10.81	0.067	
23	48.6	40.8	11.66	0.52	Start of Abrupt treatment
24	41.8	51.6	12.02	0.43	
25	34.7	52.9	11.20	0.14	
26	27.7	47.0	15.10	0.23	

SED: standard error of difference between the means

4.4.5 Effects of manipulating light to dark schedule on stress response of birds

Manipulating the light to dark schedule did not affect the stress response of birds, as measured by corticosterone concentrations in egg albumen. As shown in Figure 4-8, in all but 2 weeks of the experiment, there were no differences in the mean egg albumen corticosterone concentrations from eggs of birds in the 2 light introduction treatments.

Egg albumen corticosterone concentrations differed in weeks 20 and 21 of age (cage mean values at week 20: 1.11 vs 0.91 ng/g for the Gradual and Abrupt Light Introduction treatments, respectively; sed 0.051, P=0.003; week 21: 0.75 vs 0.67 ng/g, respectively; sed 0.028, P=0.015). The effect in the Gradual Light Introduction treatment appears to be associated with an outbreak of aggressive pecking in one cage. All birds in the cage (cage 6) were 'captured' by the stockperson and 'treated' with Stockholm tar applied to the feathers and skin, especially where the skin was lacerated and bleeding had occurred. The initial Stockholm tar treatment for cage 6 birds occurred on 10th October 2006 and the egg collections for week 20 occurred on 13th October 2006. The eggs collected from the birds in the 2 adjacent cages in the experiment, cages 5 (adjacent) and 1 (at rear), also had higher than expected egg albumen corticosterone concentrations following the Stockholm tar treatment of birds in cage 6, probably contributing to the generally elevated mean stress hormone levels in the egg albumen in the room. The birds in cage 6 were treated on 2 further occasions (16th and 17th October 2006) prior to the next egg collection date (20th October 2006 / week 21). From 20th October 2006 onwards, while Stockholm tar was applied to birds in 2 cages in the Gradual Light Introduction treatment room (cages 1 and 6) over a 10-day period, there does not appear to be a lasting response in elevated egg albumen stress hormone levels suggesting that the birds may have habituated to the treatment.

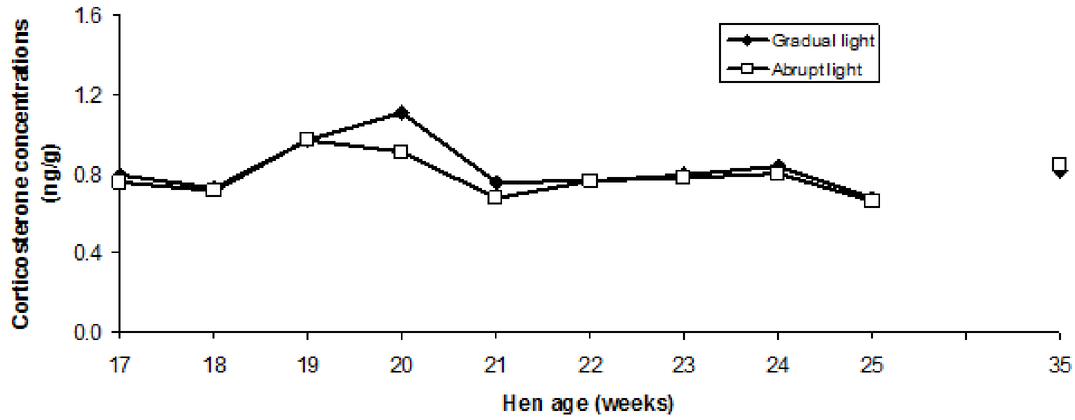


Figure 4-8- The change in mean corticosterone concentrations in the egg albumen in the Gradual and Abrupt Light Introduction treatments. Eggs were sampled on one day per week for corticosterone. Values shown are cage means. The difference detected in week 20 was was associated with an outbreak of aggressive pecking in 1 of the 2 treatments (rooms). Affected birds were treated with Stockholm tar.

4.5 Discussion

The manipulation of the light schedule, involving introduction of a period of light inserted after midnight and before 03.00 h, did not appear to affect egg production in the experiment. Regardless of the light schedule manipulation, all birds were exposed to an increasing photoperiod, culminating in a total of 16 h of light per day by 23 weeks of age, which is the recommended photoperiod to ensure good egg production in modern laying strains (Morris 2004). There have been many studies reported in the literature in which manipulating the light-dark cycle of laying hens has been investigated. Investigations have also compared various intermittent light programs, including asymmetrical patterns, symmetrical patterns with full light and symmetrical patterns with restricted light (van Tienhoven and Ostrander 1976; Morris and Bhatti 1978; Morris and Butler 1995; Morris 2004). Provided birds received a minimum of 14 h light per 24 h, interruption of the night by introducing a period of light did not affect shell breaking strength, egg size, hen day production or hen mortality. Although we did not specifically measure these parameters in the present experiment, particularly due to the limited number of birds in the experiment, there were no problems detected.

As anticipated, manipulating the light schedule in the present experiment shifted the median time of egg laying forward, coinciding with the dark period between 03.00 and 6.00 h and thus increasing the proportion of eggs laid in the dark. This was not unexpected, since the times of lights-on and lights-off are 2 key factors influencing the time of ovulation and thus oviposition in the laying hen (Morris 1973; Lewis et al. 2007a). However, the use of light introduction did not alter the synchrony of egg laying.

The method of achieving the light insertion during the dark period, that is whether it occurred gradually in 30 min increments per week over 6 weeks, or abruptly, did not result in a stress response in the birds, measured via corticosterone concentrations in eggs. An interesting finding was the stress response detected at week 20 of age. The response was associated with birds in a small number of adjacent cages in one room. The elevated corticosterone concentrations appeared to be associated with an outbreak of aggressive pecking and the subsequent handling and treatment by the stockperson. Birds were treated over a number of days with topical applications of Stockholm tar. The sensitivity of the egg albumen corticosterone assay to detect the stress response in this situation thus also validates the methodology to measure stress in laying hens.

An additional observation from this experiment was that at least some birds continued to lay in nest boxes in the dark. This was also observed in experiment 1 and has been previously reported by Sherwin and Nicol (1993a) and Appleby and Hughes (1995). As observed in experiment 1, a proportion of hens entered the nest boxes in the dark for oviposition. Other hens however, were noted to enter the nest boxes during the light and were still in the nest box when lights-off occurred, thus laying in the dark. Based on the findings of experiment 1, it can be assumed that by 18 weeks of age some birds were already consistent nest box layers, which may help explain why some birds walk to the nest box in the dark for egg laying.

Summary of findings:

- Manipulation of the light-dark schedule and abrupt versus gradual introduction of a period of light during the night, do not reduce bird welfare based on stress physiology.
- Manipulation of the light-dark schedule and the introduction of a period of light during the night altered the peak time of oviposition, coinciding with the dark period.
- Manipulation of the light-dark schedule and the introduction of a period of light during the night did not necessarily deter hens from laying in nest boxes during darkness.

5 Experiment 3 - The effects of blocking access to the nest box on the stress response of consistent nest box layers.

5.1 Background

From studies of the pre-laying activities of domestic hens, it is generally accepted there are 2 phases of behaviour involved in oviposition (Sherwin and Nicol 1992). Beginning 1 to 2 h prior to oviposition, the activity level of hens increases in a phase of behaviour termed ‘searching’ in which hens appear motivated to seek a nest site. In this phase hens increase locomotion and perform behaviours such as inspection of potential nests. Once hens have selected the preferred nest site, the ‘sitting’ phase commences. This phase includes the adoption of a sitting posture interspersed with nest-building activities such as scratching the floor/litter, rotating the body on the nest and collecting litter if available.

Activities performed in the searching phase are goal-directed or appetitive behaviours, occurring when hens are motivated to find a suitable nest for oviposition (the consummatory behaviour). Thus, Appleby and McRae (1986) and Duncan and Kite (1989) showed that hens were motivated to lay their egg in a nest box, and if a nest box was not available hens performed more nest-searching behaviour (Cooper and Appleby 1995; Freire et al. 1996). While an increased occurrence of appetitive behaviour may indicate a stronger motivation to achieve the consummatory phase, it does not necessarily indicate that increased pre-laying locomotion reflects increased frustration and thus a potential welfare problem. For example, using an aversive task approach, Freire et al. (1997) suggested that hens were only weakly motivated to reach the nest site during the searching phase, although the motivation to gain access to a nest site increased near the start of the sitting phase.

There is no doubt that laying hens have a strong preference for laying their eggs in a nest and hens are highly motivated in experimental situations to reach a familiar nest site, especially as oviposition approaches. For example, the motivation to access a secluded nest site for egg laying has been measured by how hard a bird will push through a small opening or its willingness to pass close to a dominant hen (Cooper and Appleby 1995, 1997; Freire et al. 1997, 1998).

In situ observations of pre-laying behaviour and oviposition by hens in cages with and without a nest box have been reported by many authors including Wood-Gush and Gilbert (1969), Appleby (1990), Sherwin and Nicol (1993b), Cooper and Appleby 1996, Cronin and Desnoyers (2005) and Shinmura et al. (2006a). When a nest box was unavailable, hens were more active, engaged in locomotory behaviour for a longer duration before oviposition and often performed what has been described as stereotyped pacing (Duncan and Wood-Gush, 1972; Wood-Gush 1972). Stereotyped pacing is a behavioural response that has been interpreted as a sign of frustration (Wood-Gush and Gilbert 1969; Yue and Duncan 2003; Appleby et al. 2004). Wiepkema et al. (1983) defined stereotyped pacing as a form of restless locomotion, in which the bird steps higher than normal and typically performs the action with a frantic and stereotyped character. Abnormal behaviours such as stereotyped pacing in the absence of a suitable nest site are considered by some to be behavioural pathologies and thus indicative of a welfare problem (Appleby 1998; Duncan 2001; Keeling 2004). Further, 'normal' nesting behaviour is considered essential for laying hen welfare (LayWel 2006). Although Wood-Gush (1982) later concluded that oviposition in conventional cages without a nest box leads to the performance of abnormal behaviour, he also noted that the type of abnormal behaviour shown by laying hens varied between strains and appeared to be under genetic control. Wood-Gush thus considered the possibility to breed birds that were not disturbed by the conventional cage for laying. Nevertheless, Dawkins (1990) argued that laying hens 'suffer' when deprived of suitable nest sites. Dawkins (1990) considered suffering to refer to a wide range of prolonged or acute, unpleasant subjective states (e.g. boredom, frustration, thirst). Such states appear to have evolved by natural selection as a means of avoiding danger or restoring physiological deficits resulting from an animal's natural environment. Subsequent authors including Sherwin and Nicol (1992), Duncan (1995, 2001) and Weeks and Nicol (2006) have restated Dawkin's opinion on the absence of a nest box and poor welfare, even though they state the use of nest boxes by hens can be quite variable. The finding that most hens lay in a nest box when provided is a major argument supporting the belief that a nest box is important to hen welfare (Weeks and Nicol 2006). Keeling (2004) refined this reasoning by suggesting that "if a hen is motivated to lay in a nest, but cannot find what to her is an appropriate site so, as a last resort, lays in an inappropriate place, then it probably is a welfare problem".

5.2 Objectives and hypothesis

The objectives of experiment 3 were to identify hens that were consistent nest box layers, and to measure egg production and egg albumen corticosterone concentrations of these hens in response to blocking the entrance of the nest box.

5.2.1 Hypothesis

Blocking the entrance to the nest box will elevate stress levels of consistent nest box layers.

5.3 Materials and methods

This experiment was additional to the planned project and was conducted at the conclusion of experiment 2, using 89 Hy-Line Brown birds which were then aged 39 weeks. The lighting schedule from experiment 2 was maintained in the 2 experimental rooms so that all birds were exposed to a 16 h photoperiod, with a 3 h insertion of light from midnight. Hens had been placed in the cages at 13 weeks and nest box entrances were opened at 15 weeks.

Cages of birds were allocated to treatments within rooms so that there were 3 blocked (nest box entrance blocked) and 3 control cages per room. To block the nest box entrance, a black plastic panel was inserted inside the nest box. This required about 1 min per cage and occurred between 1400-1500 h. For control treatment cages, the experimenter simulated the procedure of blocking the nest box entrance by spending the equivalent time in front of the cage and manipulating a plastic panel within the nest box before removing the panel and leaving the nest box entrance open. Eggs laid by the hens prior to, and after, nest box closure were collected for measurement of egg corticosterone concentrations. Egg corticosterone concentrations were calculated as the mean for '100% consistent' nest box layers per cage; these were determined from the video records post-hoc, based on individual birds' egg-laying site over the 7 days prior to treatment application.

Low-light video cameras were maintained in position as in experiment 2, so that a view of all birds was available from below each cage and inside each nest box. Birds were identified using a combination of black and white leg bands (as in experiment 2).

On days when eggs were to be sampled for corticosterone, the experimenter first made a grid map to show the approximate location of all eggs in each cage, viz. in the nest box, on the roll-out tray or inside the cage. The grid map also identified the cage number, date, time of collection and initials of person recording the information and a sequence number was allocated to each egg marked on the grid. The sequence number was also written in pencil on the egg (shell). Eggs were then collected and placed on trays. Once all eggs in the room had been collected, the trays of eggs were taken to the laboratory where each egg was weighed whole before being broken to separate the albumen from the yolk. The albumen was weighed then frozen for later analysis of corticosterone concentrations. The video records were examined at a later date to match eggs to the hens that laid them. The frozen albumen was stored until transported to Camden, NSW, where it remained frozen until analysed for corticosterone concentrations using the method developed by Downing and Bryden (2005). Total corticosterone concentrations in plasma were assayed using a commercial diagnostic kit (ICN ImmuChem Double antibody RIA, 7 Hills, NSW) under the supervision of Dr Jeff Downing, Faculty of Veterinary Science, University of Sydney.

5.3.1 Statistical analysis

Experiment 3 was a 2-treatment (nest box entrance Blocked vs not blocked Control), 6 replicate extended block design of 3 replicates per block design, with design blocks being rooms and plots being cages of up to 8 hens. Analysis of variance and co-variance were used to examine differences due to the treatments (GenStat 10.1, Lawes Agricultural Trust) on egg-laying characteristics and egg corticosterone concentrations. The experimental unit was the cage of birds.

5.4 Results

5.4.1 Egg production by all hens

Egg production by the 89 birds in the experiment, calculated as hen day production per treatment over the course of the experiment, is shown in Figure 5-1. Although hen day production was lower for the Blocked compared to Control treatment, the differences were not significant during either the pre-treatment (85.3 and 92.3%, respectively for the Blocked and Control treatments; sed 3.35, P=0.068) or post-treatment periods (76.1 and 90.1%, respectively; sed 7.72, P=0.104).

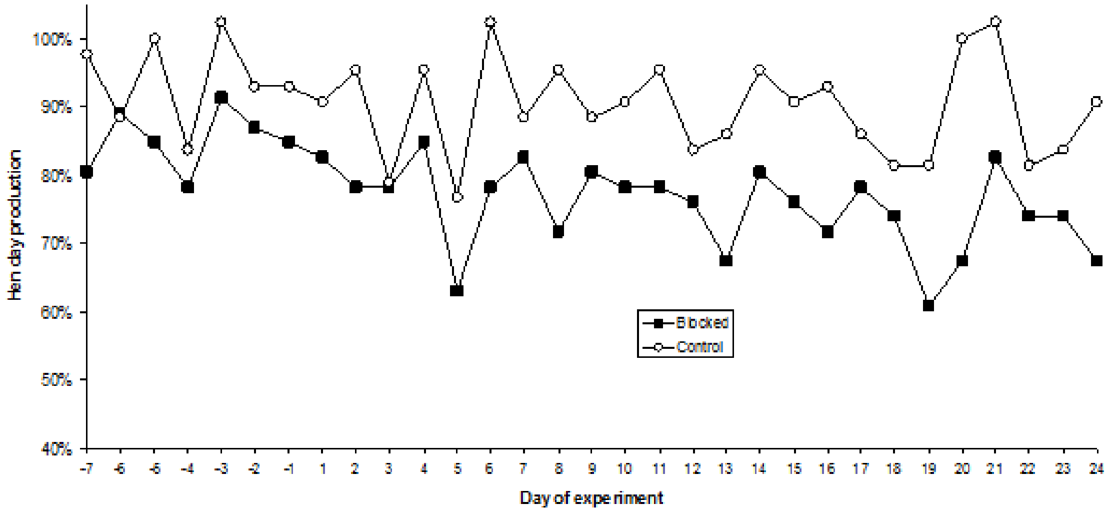


Figure 5-1 - Hen day production from cages in which the nest box was blocked or remained open (Control). The nest boxes were blocked in the early afternoon of Day -1 of the experiment. Values shown are cage means and include all eggs collected.

During the 7 days prior to nest box entrance closure, a total of 557 eggs were laid in the nest boxes (68.6% of eggs). There was a difference due to treatment in the proportion of nest box eggs recorded (61.1 and 76.4% of eggs, respectively for the Blocked and Control treatments; sed 6.19, P=0.035). Nevertheless, the majority of eggs collected in the pre-treatment period were laid in the nest boxes. The proportion of nest box eggs in the Blocked and Control treatments during the experiment are shown in Figure 5-2.

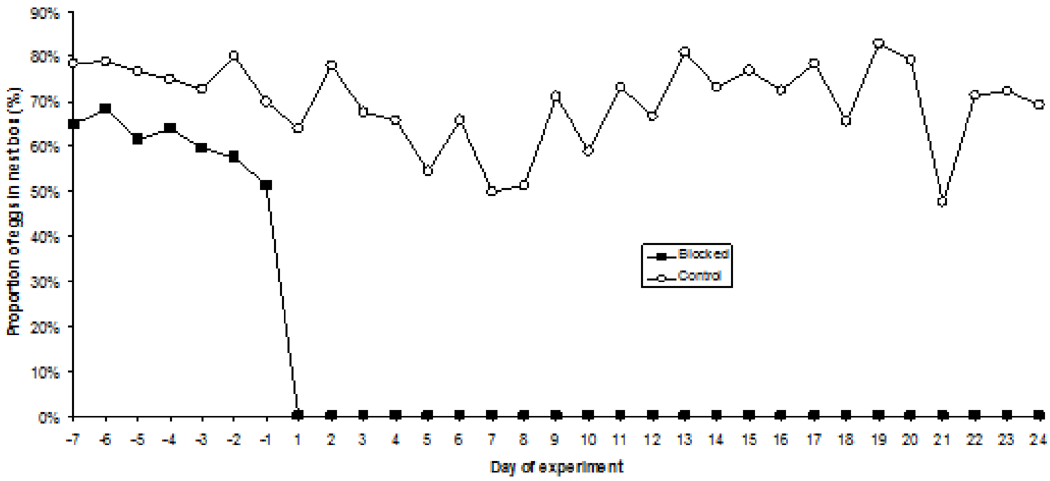


Figure 5-2 - The proportion of nest box eggs from cages in which the nest box was blocked or remained open (Control). Nest box entrances were blocked in the early afternoon of Day -1 of the experiment. Values shown are cage means and include all eggs collected.

5.4.2 Egg production by 'consistent' nest-box layers

Post-hoc examination of the video records for the 7 days prior to treatment application showed that 65.2% of hens laid at least 80% of their eggs in the nest boxes. These birds were subsequently classed as 'consistent' nest-box layers, and there were 24 and 34 hens, respectively, in the Blocked and Control treatments. Of the remaining hens, 25.8% were 100% floor layers and 9% were 'inconsistent' nest-box layers, in that they laid at least 1 egg, but less than 80% of their eggs, in the nest box over the 7 days of pre-treatment observation. The data for these birds are not included in this report.

There were no effects of treatment on the hen day egg production by 'consistent' nest-box layers following blocking or sham-blocking of nest box entrances (Figure 5-3; Table 5-1).

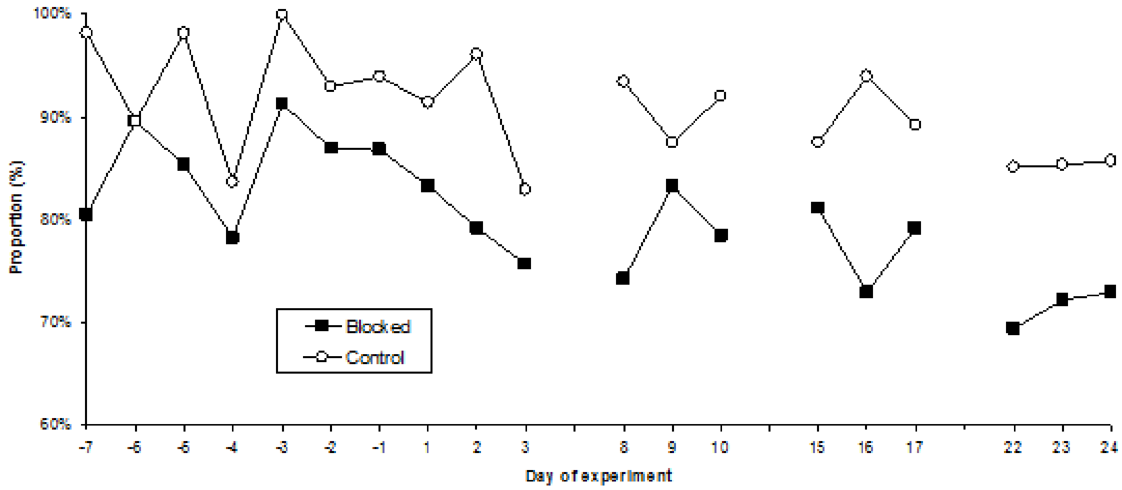


Figure 5-3 - Hen day production by hens defined as 'consistent' nest-box layers, from cages in which the nest box was blocked or remained open (Control). The nest boxes were blocked in the early afternoon of Day -1 of the experiment. Values shown are cage means. The post-treatment data shown in the figure correspond to days when eggs were collected for egg corticosterone assay.

Table 5-1 - Mean hen day egg production for ‘consistent’ nest-box layers in the Blocked and Control Treatments. Post-treatment analysis days correspond to the days when eggs were collected for corticosterone assay. Values shown are cage mean averages.

Time period	Blocked	Control	SED	P Value
Pre-treatment	89.5	88.4	3.33	0.75
Days 1 to 3	79.5	85.2	7.96	0.50
Days 8 to 10	78.1	86.8	11.04	0.45
Days 15 to 17	77.9	86.6	9.54	0.39
Days 22 to 24	70.9	84.5	11.84	0.28

SED: standard error of difference between the means

5.4.3 Corticosterone concentrations in eggs of ‘consistent’ nest-box layers

On the first 2 days post-treatment there was no difference in corticosterone concentrations in eggs between the treatments (Figure 5-4). However, corticosterone concentrations were higher on the third day in the Blocked treatment (0.82 vs. 0.69 ng/g, respectively; sed 0.054, P=0.03). Thereafter, no differences were detected.

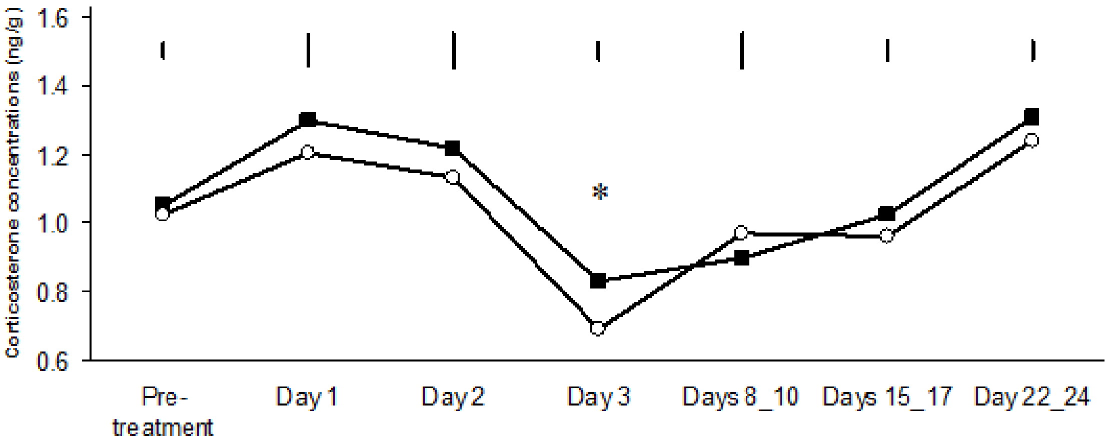


Figure 5-4 - Corticosterone concentrations in eggs after blocking (■) and sham-blocking (□) the nest box entrance. Bars represent sed. * indicates P<0.05.

5.5 Discussion

The proportion of nest box eggs recorded in this experiment (68.6%) was similar to that in our earlier experiments at the same location, but lower than in Sweden in similar cages where mean values were generally greater than 90% and often closer to 100% (Tauson and Holm 2002). The present experiment showed no effects on the number of eggs laid and only a relatively minor effect on the stress physiology of ‘consistent’ nest-box layers. For example, egg albumen corticosterone was elevated in the first 2 days after denying access to a nest box at 39 weeks, but the concentrations measured were similar to those for the sham nest box closure treatment. On the third day post-treatment, while there was a significant difference in corticosterone concentrations between the treatments, concentrations were in general at low levels.

Corticosterone transfers into albumen and accumulates during the period 3-5 h after ovulation (Downing and Bryden 2005) followed by a period of about 4 h of slow calcification when the shell membrane remains semi-permeable. Thus, the period of the day when corticosterone is more likely to enter the albumen should not coincide with the period when hens are motivated to seek a nest site for egg laying. Nevertheless, the results of experiment 2 in which the disturbance of the birds, for example due to aggressive pecking and the subsequent husbandry to treat the birds, seemed to cause prolonged elevation of corticosterone in eggs, suggests that physiological responses to environmental stressors and activation of the HPA-axis can be detected as elevated corticosterone concentrations in egg albumen. In comparison therefore, blocking the nest box entrance to preclude entry for egg laying by 'consistent' nest-box layers had little effect on the hens. Indeed, it could be argued that sham nest box entrance closure had a similar effect to permanent closure.

Overall, while most hens consistently used the nest box, there were no long-term adverse effects on stress physiology of 'consistent' nest-box layers of blocking the nest box entrance. Thus, whatever importance hens attach to a nest box, it is insufficient to result in long-term effects on the HPA axis.

Summary of findings:

- Blocking the entrance to the nest box resulted in short-term stress for consistent nest box layers, measured by elevated corticosterone concentrations in egg albumen.

6 Experiment 4 - Attractiveness of nest boxes for laying hens in cages.

6.1 Background

The welfare of laying hens housed in cages is a current international topic of ethical, political and scientific debate. In the European Union from 2013, the production of eggs from caged hens will only be acceptable if 'furniture' is provided in the cage. Furniture refers to a nest box, dust bath and perch. The focus of experiment 4 was to examine some of the factors that may influence the attractiveness of nest boxes for egg laying in young laying hens.

In AECL Project DAV-197A (Welfare of hens in furnished cages), Barnett and Cronin (2005) reported that only 62% of eggs were laid in nest boxes. A similar proportion of nest box eggs (about 70%) was recorded in experiment 1 of the present project. In the literature it is reported that hens are motivated to lay in a nest box. With the possible exception of the study by Cooper and Appleby (1997), in most experiments measuring motivation of hens to reach a nest box, hens were selected on the basis that they laid in the nest box within the experimental conditions, that is, they were 'consistent' nest box layers. Hens that chose not to lay in the nest box were excluded from the experiments. With this limitation in mind, it is perhaps not surprising that the proportion of eggs laid in nest boxes does not necessarily reflect the level of motivation of hens to use a nest box.

The incidence of floor eggs in cages with a nest box has been reported to range from 10-57% (Wall et al. 2002; Guesdon and Faure 2004; Cronin et al. 2005) but there are also reports of close to 100% nest box use in some furnished cages (Tauson and Holm 2002, 2005). In comparison, the incidence of floor eggs in an aviary system was reported to range from 0.7 and 18.4% (Abrahamsson and Tauson 1995). A number of factors have been shown to affect both the attractiveness and access to nest boxes by hens and include specific features of the nest, social factors and strain, age and rearing experience of the bird.

In furnished cages, the specific design of the nest box as well as the cage layout affect its use (Appleby et al. 2004; Barnett et al. 2005; Tauson and Holm 2005). Both the degree of seclusion and the substrate lining the nest box are important. For example, more eggs were laid in enclosed nest boxes compared with nest areas constructed of turf lined hollows in furnished cages (Appleby et al. 2004). Artificial turf is commonly used as a lining in nest boxes and has been recently shown to be as attractive as peat moss, both of which were preferred by hens over plastic coated wire mesh (Streulins et al. 2005). While more eggs are laid in nests lined with artificial turf than wire floors (Abrahamsson et al. 1996), the proportion of the nest that is lined with turf has also been shown to affect nest usage with 40.2, 26.5 and 72.4% of eggs being laid in the nest when 30, 50 or 100% of the nest box floor was lined with 'Astroturf', respectively (Wall et al. 2002), while in another study 84.8 versus 95.4% of eggs were laid in nest boxes of furnished cages with 30% versus 100% 'Astroturf' covering (Wall et al. 2002). Further, the interaction of different furnishings in cages can also affect nest box use. For example, Cronin et al. (2005) showed that nest box use increased when furnished cages also included perches.

Social factors can also affect access to a nest site (Sherwin and Nicol 1993; Friere et al. 1998) with the dominance status of hens influencing use of resources in furnished cages (Shinmura et al. 2007). During observations of hen behaviour in 25 commercial aviaries, Odén et al. (2002) reported that there was considerable aggression outside the nest boxes, suggesting competition for nest boxes. As shown in experiment 2 of this project, the majority of hens lay their eggs within a relatively short time interval during the early part of the day and therefore nest boxes should be able to accommodate multiple hens engaged in pre-laying behaviour simultaneously. Appleby et al. (2004) presented a theoretical model of nest area requirements using a minimum of 300 cm^2 of nest space per hen. Based on the probabilities of hens nesting simultaneously, the various nest area requirements of hens in different group sizes ranged from 900 cm^2 (3 nest spaces) for groups of 3 hens to 2,100 cm^2, or a total of 7 nest spaces, for a group of 12 hens.

Abrahamsson et al. (1996) and Appleby et al. (2004) reported that medium hybrid birds typically lay fewer eggs in nest boxes, an observation that corresponds to studies on the effects of strain on nesting motivation. However, all strains of birds tend to increase their use of nest boxes with age or experience over time (Sherwin and Nicol 1993; Appleby et al. 2004; results from experiments in this project). Rearing experience may also affect the use of nest boxes. For example Sherwin and Nicol (1993) found that hens reared on litter laid more floor eggs in modified cages than hens reared on wire. In non-cage systems where hens have to negotiate perches or more complex environments in order to access nest boxes, rearing in systems that encourage use of 3-dimensional space reduces floor eggs (Abrahamsson and Tauson 1995; Tauson and Holm 2005).

Even when nest designs shown to be attractive to birds are used, Sherwin and Nicol (1993) and Cooper and Appleby (1997) reported that some hens consistently chose not to lay their eggs in the nest box. Similarly, in experiment 1 of this project using a nest box that Swedish researchers reported achieved almost 100% nest box eggs, we found that only 66% of hens consistently (at least 80% of their eggs) laid in the nest box and 27% of hens consistently laid on the wire floor in an area equivalent in size to the nest box. Whether consistent floor-laying behaviour was due to a generally low motivation to use a nest box or a difference in what constitutes an attractive nest site to these particular birds is not known (Cooper and Albentosa 2003). Cooper and Appleby (1997) compared the motivation of consistent versus inconsistent nest box layers to enter a tunnel by squeezing through a narrow gap. In the tunnel, birds could perform locomotion and searching behaviour during the pre-laying period. Consistent nest box layers entered the tunnel less frequently and settled in an enclosed nest box more quickly, while inconsistent nest box layers persisted in entering the tunnel and continued searching even when a nest box was available. These authors concluded that inconsistent nest box layers were motivated to nest but their perception of what constituted a satisfactory nest differed. Similarly, while Cronin et al. (2005) found that floor layers in furnished cages stood more, walked more and entered more areas of the cage during the last 30 min before egg laying than nest box layers, it may also be considered that this difference was at least in part due to the large differences between the classes of birds in the duration of the pre-laying sitting phase that preceded oviposition. Clearly, ambient light levels moderate hens' motivation to 'search' for a suitable nest site in the first (active) pre-laying phase of egg-laying behaviour. It is known that birds are inactive if the pre-laying period coincides with darkness. Nevertheless, the lower light level inside the nest box, compared to outside, may be a relevant factor influencing the attractiveness of nest boxes for egg laying, especially for consistent nest box layers.

Social factors are also reported to influence hens' use of nest boxes. For example, Shinmura et al. (2007) reported that dominant birds were more likely to lay in nest boxes in furnished cages than subordinate hens. Hen postures such as standing erect and holding the head high are signals used by hens to display dominance. Less dominant hens signal their subordinate position in the social hierarchy by lowering their posture and their head in the presence of a dominant bird (McBride et al. 1969). The design of nest box used in the present project required birds to lower their head and stoop their posture in order to enter the nest box. Thus, it is possible that certain birds may not wish to signal a low dominance position by stooping to enter the nest box. If so, these birds may prefer to remain outside the nest box for egg laying, becoming floor layers.

Experiment 4 aimed to investigate why about one-third of hens in our previous experiments at Werribee, using Victorsson furnished cages and Hy-Line Brown birds, chose to lay on the wire cage floor rather than in a nest box. The final experiment consisted of 3 short experiments focussing on young hens at the commencement of, and during the early stages of, egg laying. The 3 parts of the experiment were designed to investigate the effects of (a) light levels inside the nest box, (b) the h8 of the nest box entrance and interior nest box space and (c) social group size, on utilisation of nest boxes during early lay. The rationale for part (a) was to determine whether provision of more light in the nest box, as would occur if the entrance was taller, would alter hens' preference for laying inside compared to outside the nest box. Provided no difference was detected in part (a), part (b) would then investigate the effects of modifying the h8 of the nest entrance and the inside of the nest box so that hens could enter the nest box without stooping. Finally, part (c) investigated the effects of social competition for the nest box by housing birds either singly (ie. 1 nest box per bird) or in groups of 8 (ie. 1 nest box for 8 birds).

6.2 Experiment 4A - The effects of light level inside the nest box on the proportion of nest box and floor eggs.

6.2.1 Objectives and hypothesis

The objective of this experiment was to investigate the effect of light level inside the nest box on the proportion eggs laid in the nest box during the early stage of lay. If an effect of higher lux was not found, then the next experiment could proceed in the knowledge that additional light entering the nest box should not be a confounding factor. The subsequent experiment was planned to investigate the effect of increasing the h8 of the interior of the nest box, including the h8 of the nest box entrance, on proportion of eggs laid in the nest box.

6.2.1.1 Hypothesis

Increased light level inside the nest box reduces the proportion of eggs laid in the nest box.

6.2.2 Materials and methods

A total of 96 Hy-line Brown hens were housed at 8 birds per cage in 12 Victorsson Trivselburen furnished cages, modified by removing the perch and dust bath. All cages contained a nest box located on the right side of the cage (viewed from the front) and measured 1.2 m wide, 0.5 m deep and 0.45 m high at the rear of the cage. The nest box, which measured 0.24 m wide, 0.5 m deep and 0.27 m high at the front of the cage, had a solid ceiling, rear and sides, apart from an entrance opening in 1 side wall. A blue vinyl flap covered the front of the nest box while the nest box floor was overlain with 'Astro turf' (0.37 m x 0.22 m x 15 mm thick). The cages were located within an insulated poultry shed divided into 2 experimental rooms to prevent entry of external light while allowing control over air temperature and ventilation. Each experimental room contained a bank of cages. These banks of cages contained 10 cages per tier, arranged with 2 cages back-to-back and 5 cages side-by-side. The 2 tiers (ie. the upper and lower tiers) were separated by a vertical space equivalent to the h8 of a cage. 6 cages in the upper tier of each bank were used in the experiment. The experiment commenced in May 2007. At 13 weeks of age the birds were transported to Werribee, beak trimmed, identified by leg rings and placed at random in the observation cages. The nest boxes were closed at this time and remained closed to the birds for the first 2 weeks in the cages. At entry to the cages, birds were exposed to a 12L:12D light schedule, which was increased to 16L:8D by 24 weeks of age. There were 2 treatments:

Light in nest box treatment – a single white light globe (part of a Christmas light set) was placed inside the nest box, just below the ceiling at the rear of the nest box. The light was controlled by a timer that switched the light on and off in concert with the room lights. The illumination level inside the nest box when the light was on was about 4 lux at the front of the nest box.

Control treatment – a single globe (part of a Christmas light set), not connected to power and thus not illuminated, was placed inside the nest box, just below the ceiling at the rear of the nest box. The illumination level inside the nest box was about 2 lux at the front of the nest box.

Treatments were allocated at random within rooms, so that within each room there were 3 cages of each treatment. The experimenters recorded the location of all eggs on a grid map for each cage before eggs were collected each day. 5 areas across the cage were recognised, with each area being equivalent to the area of the nest box, which occupied one-fifth of the cage. Data were collated and the differences due to the treatments, blocked on room, on the number of eggs laid and proportion laid in the nest box were analysed using Genstat 10.1. The experimental unit was the cage average.

6.2.3 Results

There was no difference in mean age at first egg recorded per cage for the Light and Control treatments (113.8 days; sed 1.54, P=1.0). Similarly, hen day egg production did not differ due to the treatments during the period 15-19 weeks of age (39.6 and 39.1%, respectively for the Light and Control treatments; sed 2.95, P=0.47) or 20-26 weeks (90.8 and 96.2%, respectively; sed 3.57, P=0.17). Figure 6-1 shows the change in hen day egg production per week for the 2 treatments over the period of the experiment.

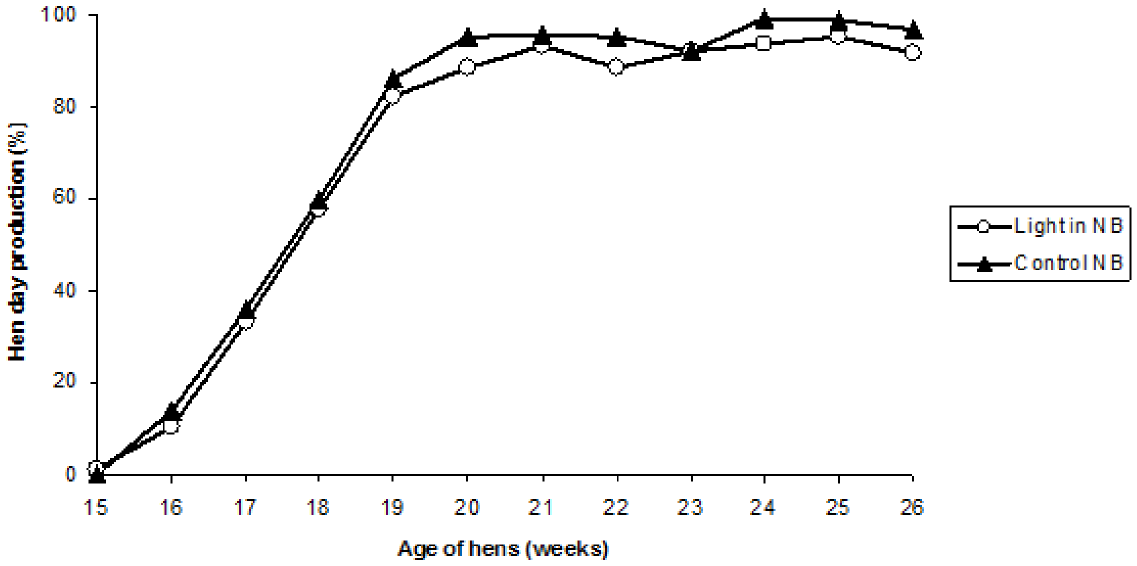


Figure 6-1 - Hen day egg production per week for the Light in nest box (NB) and Control treatments. The values shown are cage averages.

Similarly, the proportion of eggs laid in the nest boxes did not differ due to the treatments during the period 15-19 weeks of age (47.7 and 49.8%, respectively for the Light and Control treatments; sed 8.41, P=0.80) or 20-26 weeks (79.7 and 76.5%, respectively; sed 9.44, P=0.74). The change in the proportion of nest box eggs is shown in Figure 6-2. While there was no effect of treatment on the proportion of nest box eggs (P>0.05), the proportion of nest box eggs in this experiment was the highest recorded in the series of experiments in this 3 year project. In the period weeks 20 to 26 of age, 78.1% of eggs (pooled mean) were recorded as being laid in the nest boxes.

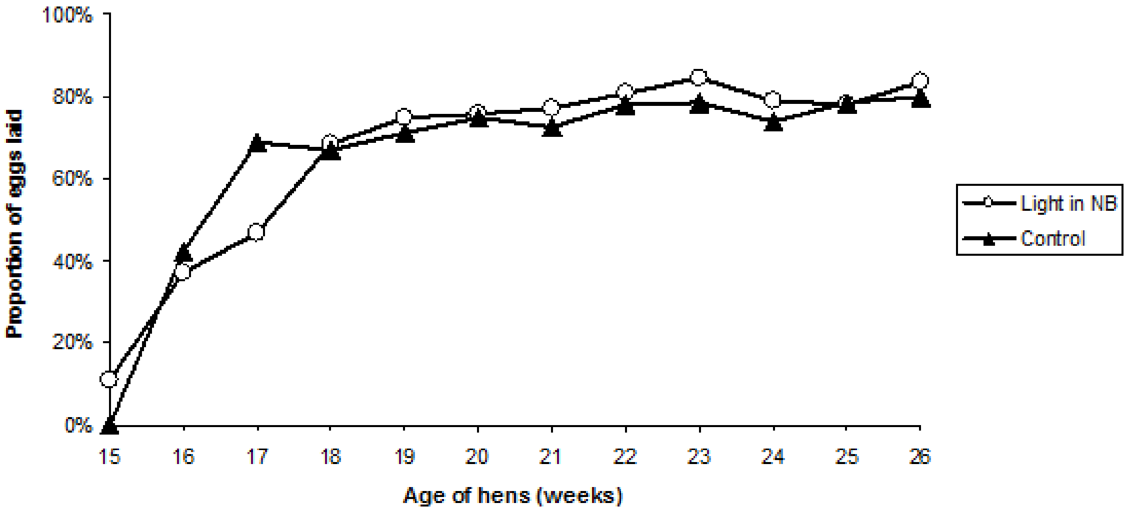


Figure 6-2 - The proportion of eggs laid per week in the nest box (NB) by birds in the Light in NB and Control treatments. The values shown are cage averages.

A post-hoc analysis of the data was conducted to determine whether there were differences due to the experimental rooms, as differences were found between the 2 rooms in the third small experiment in this series in hen day egg production and the proportion of nest box eggs. While no significant differences were found in any of the parameters measured, there was a weak effect (P=0.071) of room on age at first egg recorded per cage (112.2 and 115.5 days of age, respectively for birds in Room 1 and 2; sed 1.60). Changes in hen day production and the proportion of nest box eggs per week for birds in the Light and Control treatments in the 2 experimental rooms are shown in Figures 6-3 and 6-4, respectively.

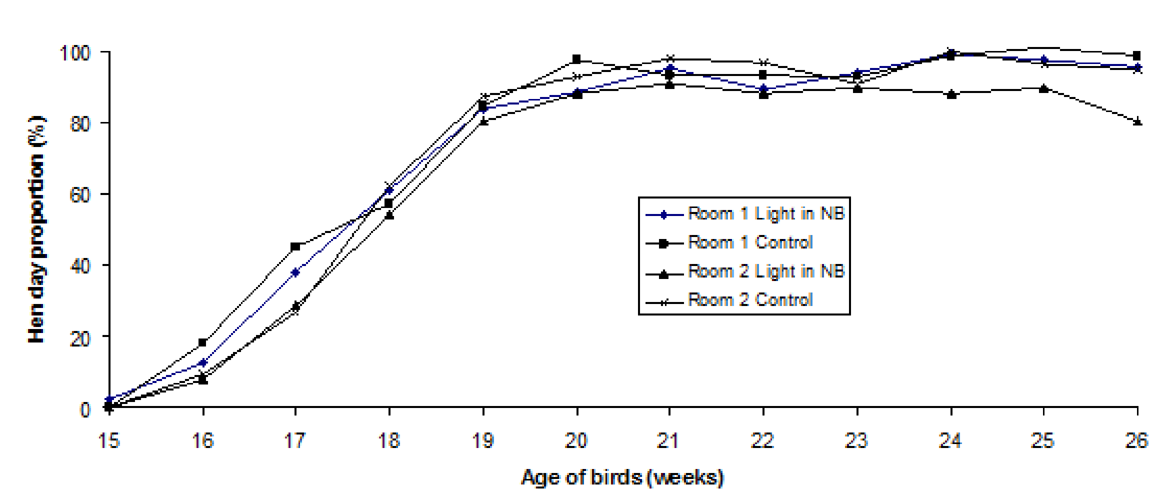


Figure 6-3 - Egg production per week by birds in the Light in Nest Box (NB) and Control treatments in the 2 experimental rooms. The values shown are cage averages.

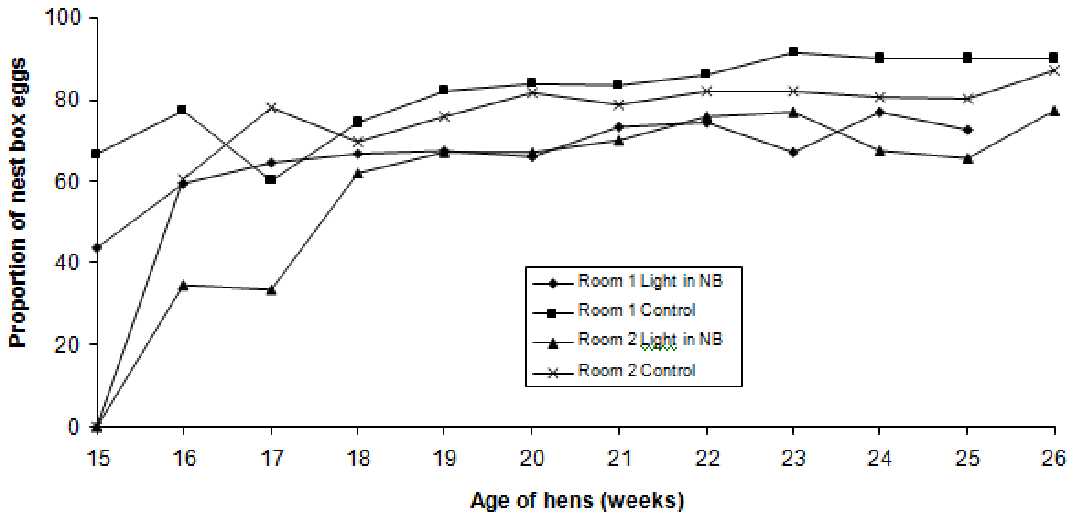


Figure 6-4 - The proportion of eggs laid per week in the nest box (NB) by birds in the Light in NB and Control treatments in the 2 experimental rooms. The values shown are cage averages.

6.2.4 Discussion

There were no effects of increasing the light level in the nest boxes from 2 to 4 lux on either egg production or the proportion of nest box eggs. It was therefore feasible to proceed with the next experiment to investigate the effects of nest box (entrance) h8 on the incidence of nest box eggs.

One interesting finding in the present experiment was that the proportion of eggs laid in the nest boxes, pooled for the 2 treatments, approached 80% of eggs laid. This was in fact the highest proportion of nest box eggs we recorded in our experiments conducted at the Werribee site for hens in groups in cages. The small (shiny) light bulb per se, placed inside each nest box, regardless of whether the bulb was lit, may have been attractive to the hens and encouraged them to enter the nest boxes. Thus, the bulbs may have facilitated the young hens becoming familiar with the nest boxes before reaching point of lay. The concept of adding an attraction inside the nest box may be worth pursuing at a later opportunity.

Finding:

- Increased light level inside the nest box did not reduce the proportion of eggs laid in the nest box.

6.3 Experiment 4B - The effects of the internal h8 of the nest box and the nest box entrance on the proportion of nest box and floor eggs.

6.3.1 Objectives and hypothesis

The objective of the second small experiment in this series was to investigate the effects of h8 of the nest box interior, including the h8 of the entrance, on the proportion eggs laid in the nest box by hens during the early stage of lay.

6.3.1.1 Null hypothesis

- Increased h8 of the nest box does not reduce the proportion of eggs laid in the nest box.

6.3.2 Materials and methods

A total of 96 Hy-line Brown hens were housed at 8 birds per cage in 12 Victorsson Trivselburen furnished cages, modified by removing the perch and dust bath. All cages contained a nest box located on the right side of the cage (viewed from the front) and measured 1.2 m wide, 0.5 m deep and 0.45 m high at the rear of the cage. The nest box, which measured 0.24 m wide, 0.5 m deep and 0.27 m high at the front of the cage, had a solid ceiling, rear and sides, apart from an entrance opening in 1 side wall. A blue vinyl flap covered the front of the nest box while the nest box floor was overlain with 'Astro turf' (0.37 m x 0.22 m x 15 mm thick). The cages were located within an insulated poultry shed divided into 2 experimental rooms to prevent entry of external light while allowing control over air temperature and ventilation. Each experimental room contained a bank of cages. These banks of cages contained 10 cages per tier level, arranged with 2 cages back-to-back and 5 cages side-by-side. The 2 tiers (ie. the upper and lower tiers) were separated by a vertical space equivalent to the h8 of a cage. 6 cages in each upper tier were used in the experiment. The experiment commenced in September 2007. At 13 weeks of age the birds were transported to Werribee, beak trimmed, identified by leg rings and placed at random in the observation cages. The nest boxes were closed at this time and remained closed to the birds for the first week in the cages. At entry to the cages, birds were exposed to a 12L:12D light schedule, which was increased to 16L:8D by 24 weeks of age. There were 2 treatments:

Tall nest box treatment – the h8 of the nest box, including the entrance to the nest box, was increased to the full h8 of the cage (0.5 m at the cage front and 0.45 m at the cage rear). The nest box had a solid ceiling.

Control nest box treatment – the 'standard' Victorsson nest box, which was 0.27 m high at the front of the cage, was used.

Treatments were allocated at random within rooms, so that within rooms there were 3 cages of each treatment. The experimenters recorded the location of all eggs on a grid map before eggs were collected each day. 5 areas across the cage were recognised, with each area being equivalent to the area of the nest box, which occupied one-fifth of the cage. Data were collated and the differences due to the treatments and rooms on the number of eggs laid and proportion laid in the nest box were analysed using Genstat 10.1. The experimental unit was the cage of birds.

6.3.3 Results

As in the previous small experiment, there was no difference in mean age at first egg recorded per cage for the Tall and Control nest box treatments (112.0 and 110.5 days, respectively; sed 1.92, P=0.45). Similarly, hen day egg production did not differ due to the treatments during the period 15-19 weeks of age (44.2 and 46.7%, respectively for the Tall and Control nest box treatments; sed 7.43, P=0.47) or 20-26 weeks (88.1 and 87.3%, respectively; sed 1.74, P=0.66). Figure 6-5 shows the change in hen day egg production per week for the 2 treatments over the period of the experiment.

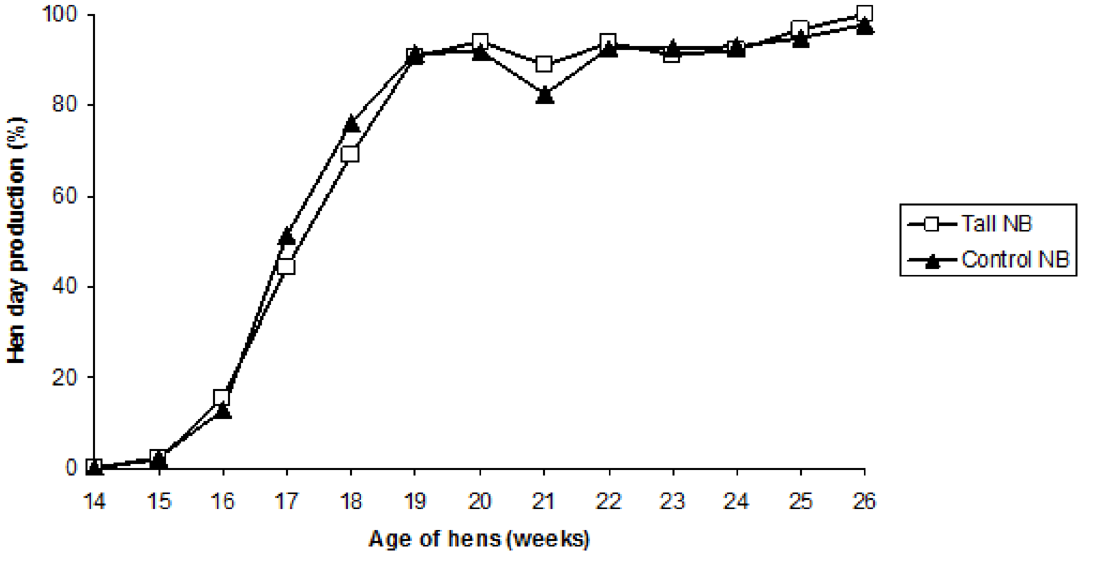


Figure 6-5 - Hen day egg production per week in the Tall and Control nest box (NB) treatment cages. The values shown are cage averages.

Similarly, the proportion of eggs laid in the nest boxes did not differ due to the treatments during the period 15-19 weeks of age (43.4 and 41.2%, respectively for the Tall and Control nest box treatments; sed 9.62, P=0.82) or 20-26 weeks (58.4 and 52.7%, respectively; sed 10.33, P=0.60). The change in the proportion of nest box eggs is shown in Figure 6-2. There was no effect of treatment on the proportion of nest box eggs (P>0.05).

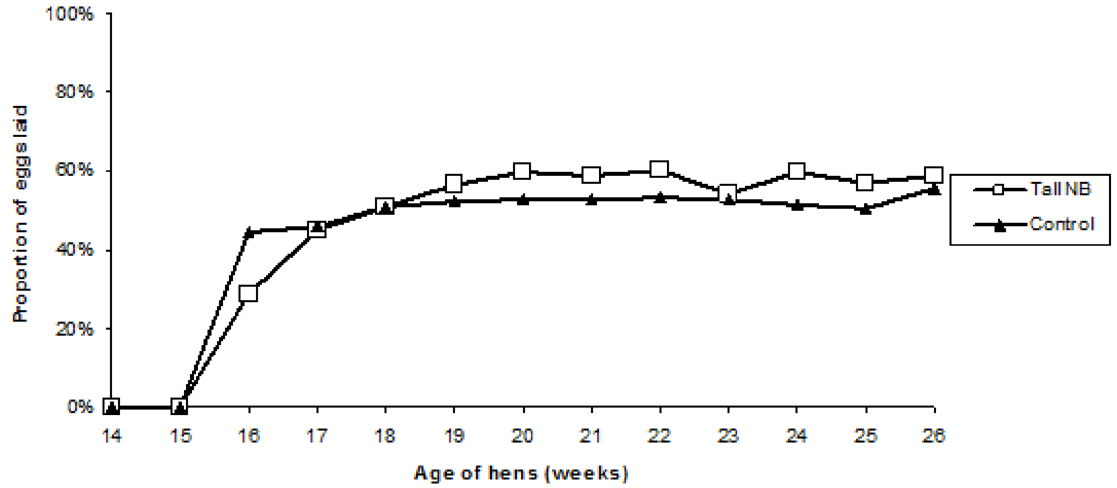


Figure 6-6 - The proportion of eggs laid per week in the Tall and Control nest box treatment cages. The values shown are cage averages.

As in the previous experiment, a post-hoc analysis of the data was conducted to determine whether there were effects of the experimental rooms, as differences were found in the third experiment in the proportion of nest box eggs between the 2 rooms. There were no significant differences found in any of the parameters measured. Changes in hen day production and the proportion of nest box eggs per week for birds in the Tall nest box and Control treatments in the 2 experimental rooms are shown in Figures 6-7 and 6-8, respectively.

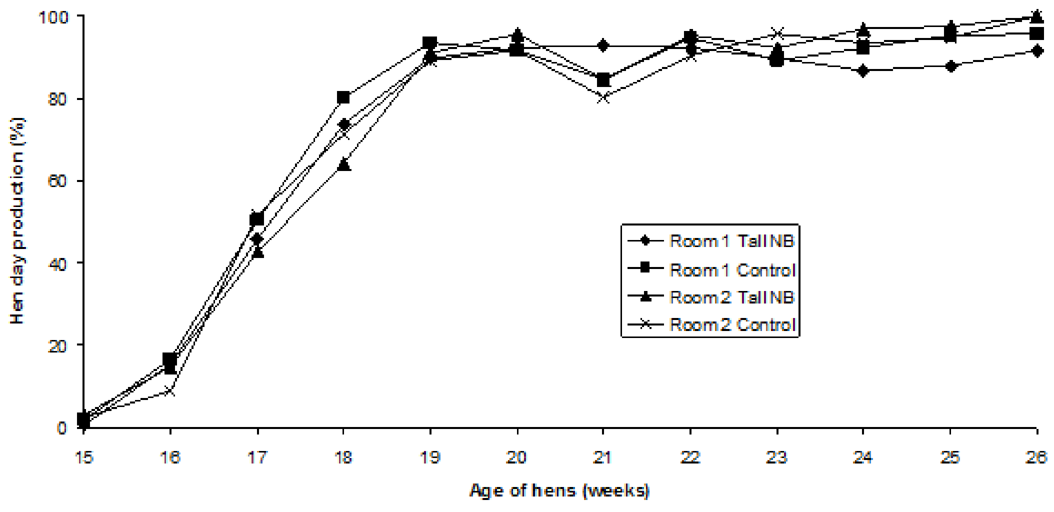


Figure 6-7 - Hen day egg production per week by birds in the Tall and Control nest box treatment cages in the 2 experimental rooms. The values shown are cage averages.

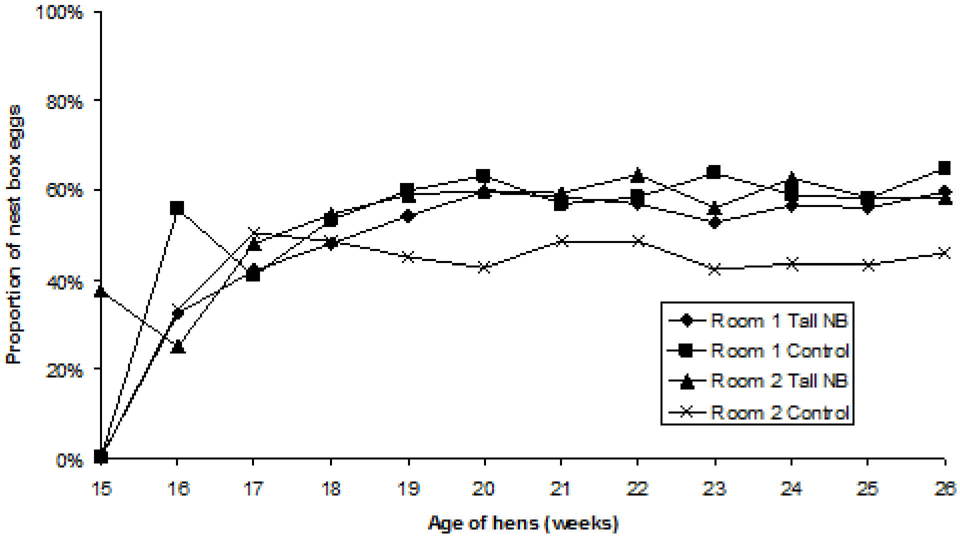


Figure 6-8 - The proportion of eggs laid in the nest box (NB) per week by birds in the Tall and Control nest box treatment cages in the 2 experimental rooms. The values shown are cage averages.

6.3.4 Discussion

The overall proportion of nest box eggs in this small experiment was relatively low (55% of eggs). Nevertheless, there were no effects of increasing the h8 of the nest boxes, including the entrances to the nest boxes, on either egg production or nest box use. Although the difference was not significant, the proportion of nest box eggs was lower in Room 2, particularly due to the Control treatment in which only 45% of eggs were laid in nest boxes. A larger experiment would be required to determine whether there were real effects of room or an interaction between the treatment and room on nest box use.

Finding:

- Increased h8 of the nest box (and the entrance) did not reduce the proportion of eggs laid in the nest box.

6.4 Experiment 4C - The effects of social group size on the proportion of nest box and floor eggs.

6.4.1 Objectives and hypothesis

The objective of this experiment was to investigate the effects of social group size on the proportion of eggs laid in the nest box by hens during the early stage of lay.

6.4.1.1 Hypothesis

- Group housed compared to single housed hens lay proportionally fewer eggs in the nest box.

6.4.2 Materials and methods

A total of 96 Hy-line Brown hens were housed either singly (ie. 1 bird per cage) or at 8 birds per cage in Victorsson Trivselburen 8-bird furnished cages, modified by removing the perch and dust bath. All cages contained a nest box located on the right side of the cage (viewed from the front) and measured 1.2 m wide, 0.5 m deep and 0.45 m high at the rear of the cage. The nest box, which measured 0.24 m wide, 0.5 m deep and 0.27 m high at the front of the cage, had a solid ceiling, rear and sides, apart from an entrance opening in 1 side wall. A blue vinyl flap covered the front of the nest box while the nest box floor was overlain with 'Astro turf' (0.37 m x 0.22 m x 15 mm thick). The cages were located within an insulated poultry shed divided into 2 experimental rooms to prevent entry of external light while allowing control over air temperature and ventilation. Each experimental room contained a bank of cages with 10 cages per tier, arranged with 2 cages back-to-back and 5 cages side-by-side. The 2 tiers (ie. the upper and lower tiers) were separated by a vertical space equivalent to the h8 of a cage. Both tiers of each bank were used in the experiment, providing a total of 40 cages.

The experiment commenced in March 2008. At 13 weeks of age the birds were transported to Werribee, beak trimmed, identified by leg rings and placed at random in the observation cages. The nest boxes were closed at this time and remained closed to the birds for the first 2 weeks in the cages. At entry to the cages, birds were exposed to a 12L:12D light schedule, which was increased to 16L:8D by 24 weeks of age.

There were 2 treatments:
Single bird per cage treatment – The cage contained 1 bird.
8 birds per cage treatment – The cage contained 8 birds.

Treatments were allocated at random to tiers within rooms, so that within each tier there were 8 cages containing single birds and 2 cages of 8 birds. The experimenters recorded the location of all eggs on a grid map for each cage before eggs were collected each day. 5 areas across the cage were recognised, with each area being equivalent to the area of the nest box, which occupied one-fifth of the cage. Data were collated and differences due to the treatment, room and tier on the number of eggs laid and proportion laid in the nest box were analysed using Genstat 10.1. The experimental unit was the cage.

6.4.3 Results

Hen day egg production did not differ between the group size treatments during the period 19-24 weeks of age (88.5 and 90.8%, respectively for the 1-bird and 8-bird treatments; sed 4.24, P=0.58). Figure 6-9 shows the change in hen day egg production per week for the 2 treatments over the period of the experiment.

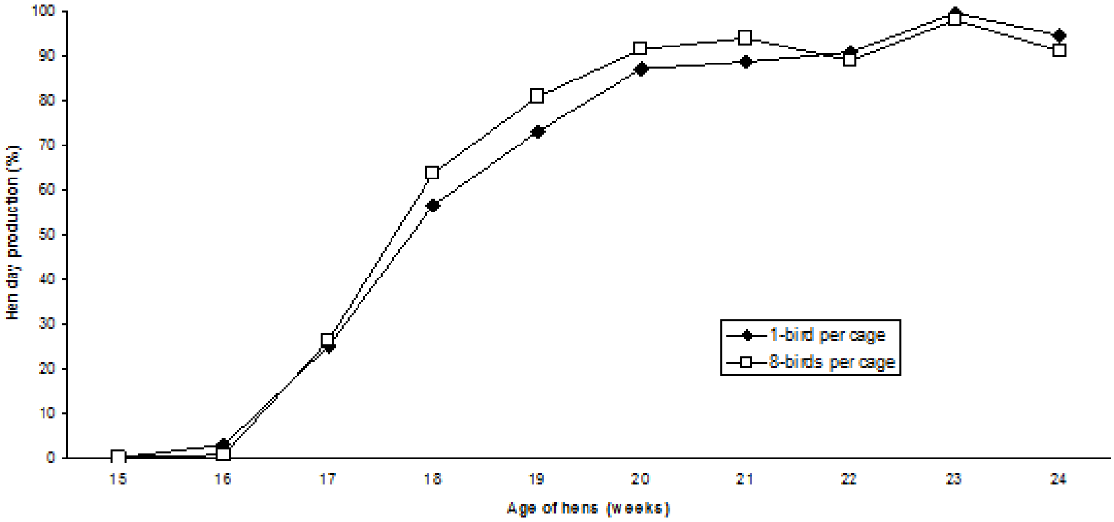


Figure 6-9 - Hen day egg production per week in the Single bird and Group (8 birds per cage) treatments. The values shown are cage averages.

Although the proportion of eggs laid in the nest box was greater on average for the 1-bird compared to the 8-bird treatment, the difference was not significant during the period 19-24 weeks of age (79.9 and 69.3%, respectively for the 1-bird and 8-bird treatments; sed 11.13, P=0.34). Figure 6-10 shows the change in hen day egg production per week for the 2 treatments over the period of the experiment.

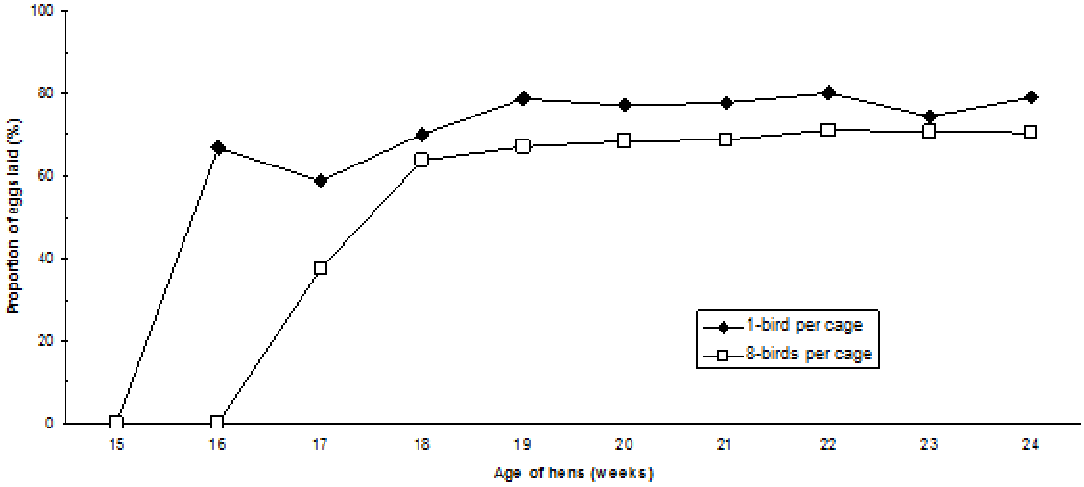


Figure 6-10 - The proportion of eggs laid in the nest box per week in the Single bird and Group (8 birds per cage) treatments. The values shown are cage averages.

Between 19 and 24 weeks of age, hen day egg production was found to be greater in Room 1 than Room 2 (94.7 and 83.2%, respectively; sed 3.33, P=0.002). This difference was largely due to lower egg production from 1-bird cages in Room 2 (Figure 6-11). Further, the proportion of eggs laid in nest boxes during the same period was greater in Room 1 than Room 2 (89.9 and 65.7%, respectively; sed 8.94, P=0.010). As shown in Figure 6-12, there was a very high proportion of nest box eggs in 1-bird cages in Room 1 (93.3%). The proportion of nest box eggs in 8-bird cages in Room 1 was 76.3%. In comparison, in Room 2, the proportion of nest box eggs was similar in both group size treatments (1-bird cages 66.6%; 8-bird cages 62.2%). While there were significant effects of room on both parameters, there were no significant interactions between the group size treatments and room, and there were no effects of tier on any of the variables measured.

Changes in hen day egg production and the proportion of nest box eggs per week for birds in the 1-bird and 8-bird treatments in the 2 experimental rooms are shown in Figures 6-11 and 6-12, respectively.

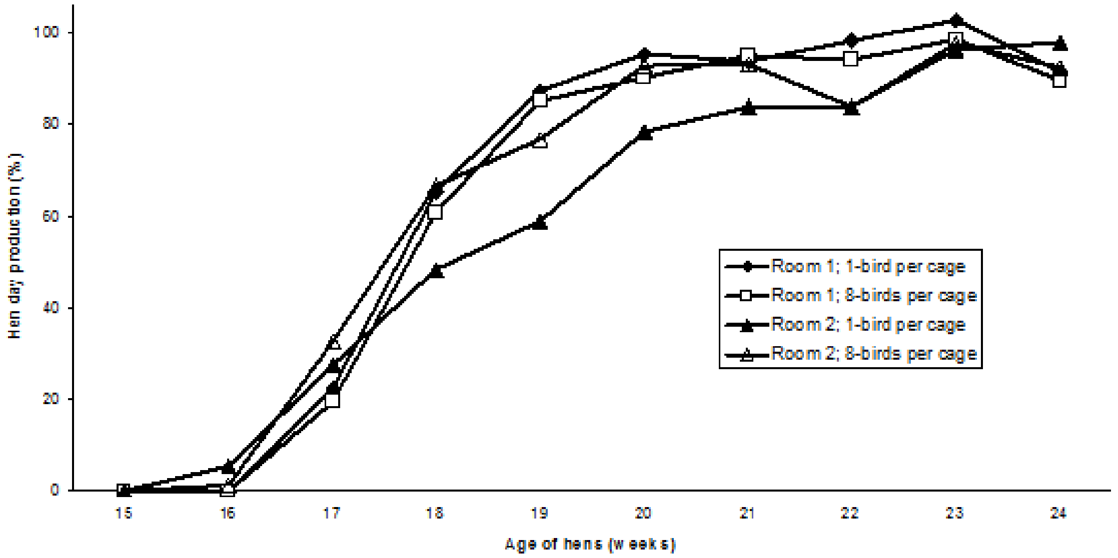


Figure 6-11 - The effects of Group size and Room on hen day egg production. Values shown are weekly means based on cage averages.

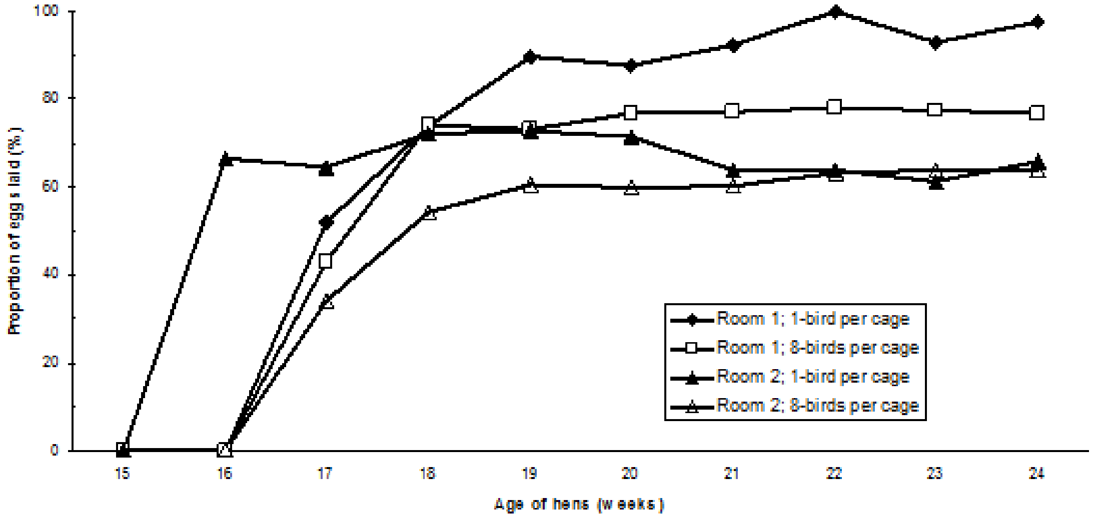


Figure 6-12 - The effects of Group size and Room on the proportion of eggs laid in the nest box. Values shown are weekly means based on cage averages.

The proportion of nest box eggs laid per hen in the 2 treatments in each room is shown in Figure 6-13. As indicated in the figure, 12 birds in the 1-bird cages were 100% consistent nest box layers, while 1 bird in a 1-bird cage in Room 2, did not lay any of her eggs in the nest box (ie. 100% floor layer).

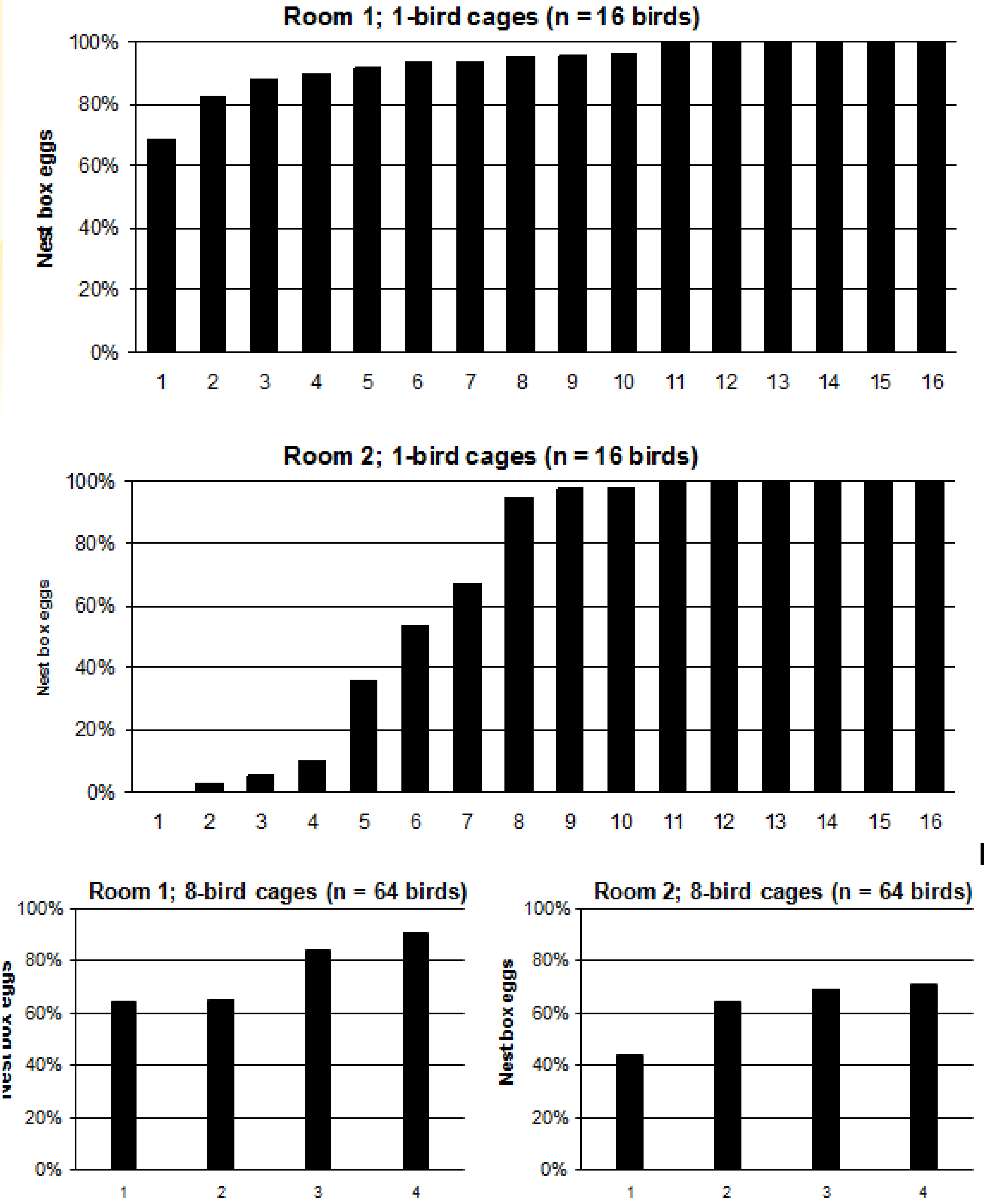


Figure 6-13 - Frequency distributions showing the proportion of eggs laid in the nest boxes by hens in 1-bird and 8-bird cages.

6.4.4 Discussion

The proportion of nest box eggs laid in the single bird cages in Room 1 was very high (93.3% of eggs), and 15 of the 16 birds laid >80% of their eggs in the nest boxes, indicating they were consistent nest box layers. In comparison, in the 8-bird cages in Room 1, about 3 quarters of eggs (76.3%) were laid in the nest boxes. While the data for individual birds in the 8-bird cages was not recorded, Figure 6-13 suggests that the majority of birds in at least 2 of the 4 8-bird cages were consistent nest box layers.

The proportion of nest box eggs recorded in Room 2 was comparatively low (about 2-thirds of eggs laid) than for Room 1. 4 (25%) of the birds in 1-bird cages would be classed as consistent floor layers (>80% eggs laid outside the nest boxes), including 1 bird that laid all her eggs outside the nest box.

A small temperature difference existed between the rooms, with Room 1 being about 1ºC cooler than Room 2. While this temperature difference may be relevant to egg production, how such a small temperature differential could impact on nest box use is unclear. Nevertheless, ambient temperature and thermal comfort may be factors contributing to the motivation of birds to use nest boxes for egg laying.

The finding that 93% of eggs were laid in the nest box of 1-bird cages in Room 1 compared to 76% in 8-bird cages, supports the contention that social factors may adversely influence nest box use.

Findings:

- Group housed compared to single housed hens laid proportionally fewer, although not significantly fewer, eggs in the nest box.
- Differences in egg production and nest box use detected between the rooms may be associated with differences in the thermal environment.

Summary of findings:

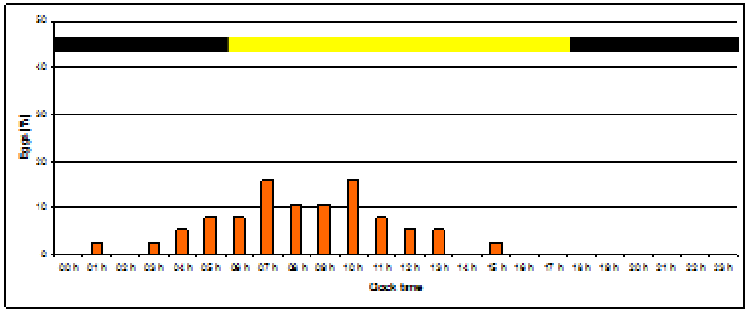
- Increasing the level of light from 2 to 4 lux inside the nest box did not alter the proportion of nest box eggs.
- Increasing the h8 of the entrance to the nest box, and the internal h8 of the nest box, did not alter the proportion of eggs laid in the nest box.
- Group housed compared to single housed hens laid proportionally fewer, although not significantly fewer, eggs in the nest box.
- Differences in egg production and nest box use detected between the rooms may be associated with differences in the thermal environment.

7 Appendix I

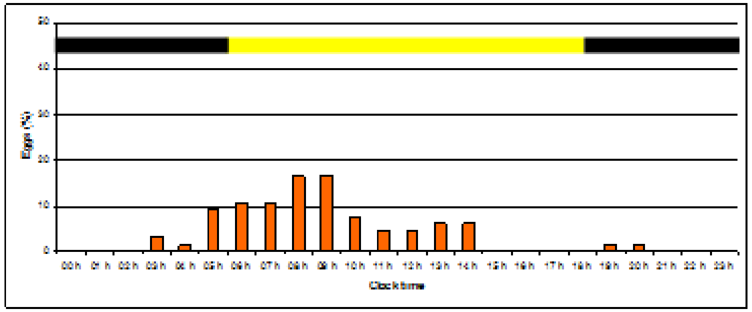
Appendix I – Distribution graphs (vertical bars) showing the proportion of eggs laid per hour during 4 days per week, for birds exposed to the 2 light introduction treatments. Photoperiod is represented by the horizontal bar above each graph (Light (L) – yellow; Dark (D) – black).

Gradual Light Increase Treatment

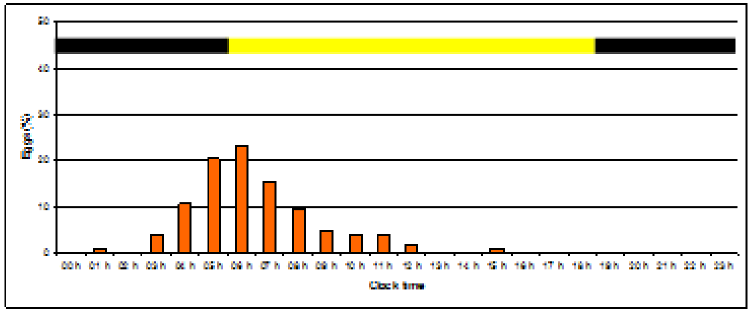
Hen age 15 wks; N=38 eggs; 12 L : 12 D



Hen age 16 wks; N=66 eggs; 12.5 L : 11.5 D

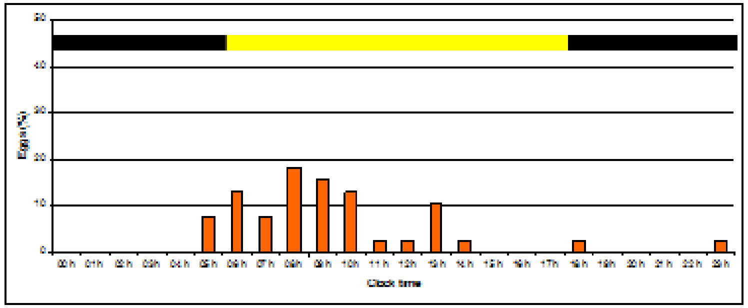


Hen age 17 wks; N=103 eggs; 13 L : 11 D

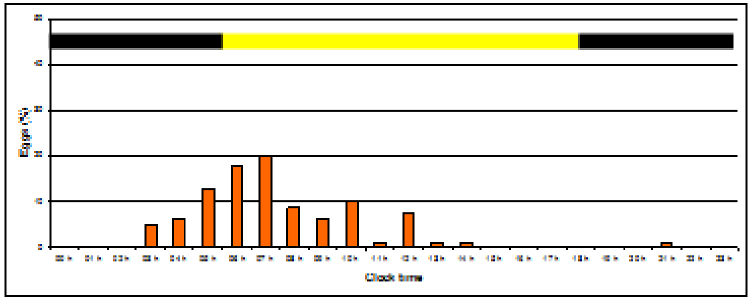


Abrupt Light Increase Treatment

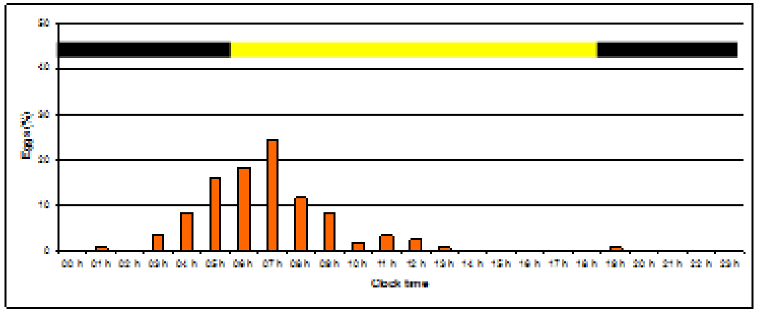
Hen age 15 wks; N=38 eggs; 12 L : 12 D



Hen age 16 wks; N=79 eggs; 12.5 L : 11.5 D

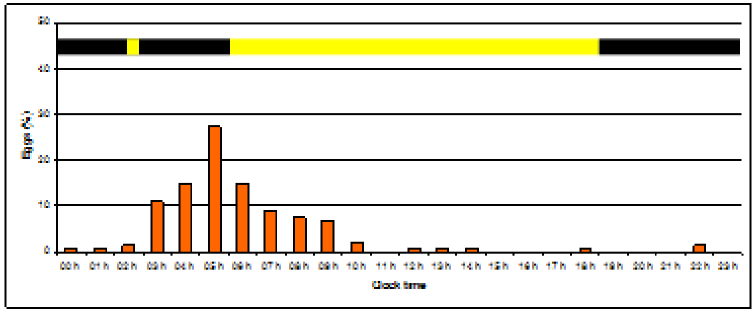


Hen age 17 wks; N=120 eggs; 13 L : 11 D

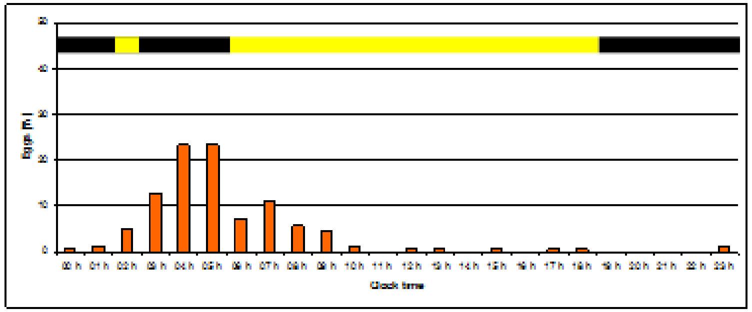


Gradual Light Increase Treatment

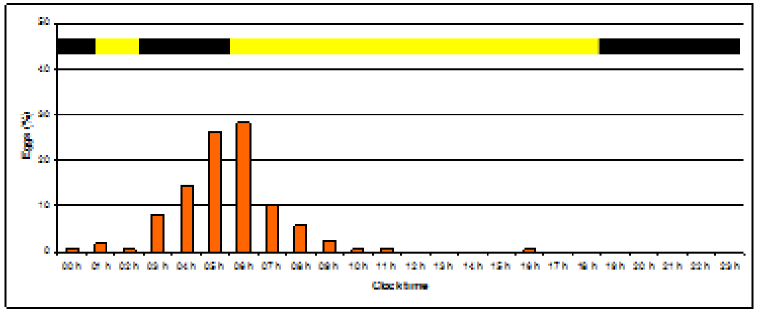
Hen age 18 wks; N=147 eggs; 13 L ; 7.5 D : 0.5 L : 3 D



Hen age 19 wks; N=179 eggs; 13 L ; 7 D : 1 L 3 D

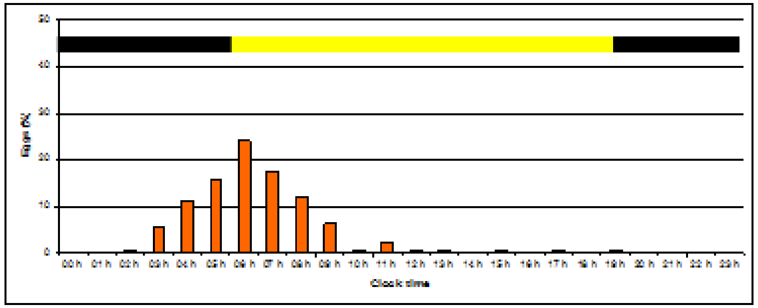


Hen age 20 wks; N=171 eggs; 13 L ; 6.5 D : 1.5 L : 3 D

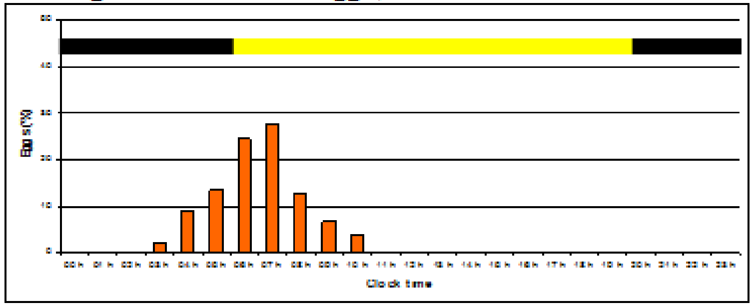


Abrupt Light Increase Treatment

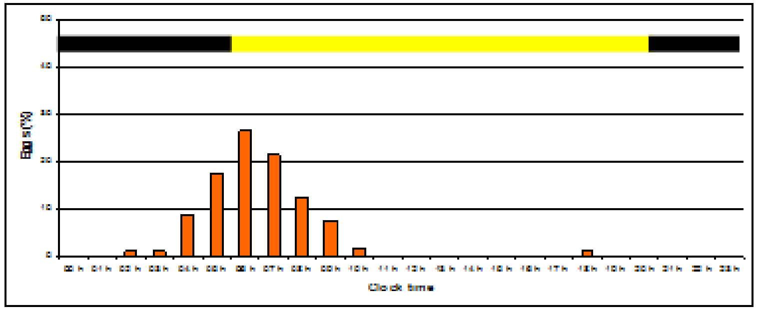
Hen age 18 wks; N=158 eggs; 13.5 L : 10.5 D



Hen age 19 wks; N=148 eggs†; 14 L : 10 D



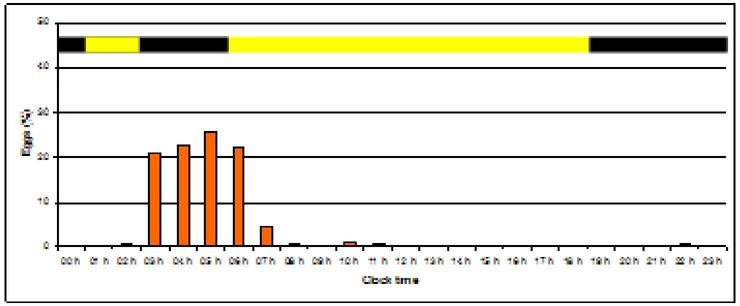
Hen age 20 wks; N=181 eggs; 14.5 L : 9.5 D



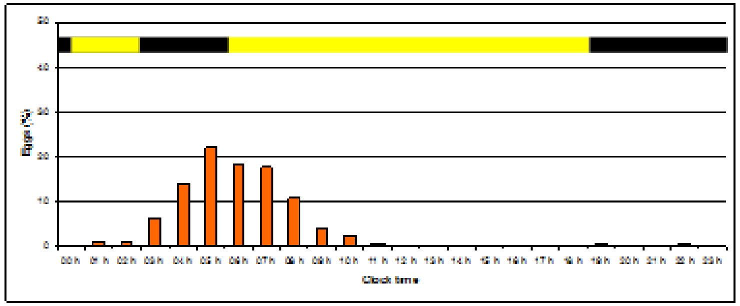
† video system failure reduced data collection in the week

Gradual Light Increase Treatment

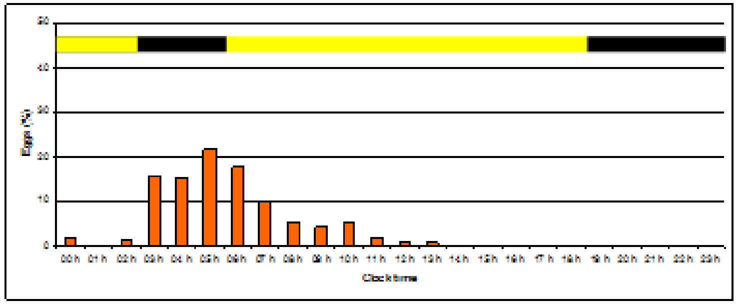
Hen age 21 wks; N=175 eggs; 13 L : 6 D : 2 L : 3 D



Hen age 22 wks; N=158 eggs†; 13 L : 5.5 D : 2.5 L : 3 D

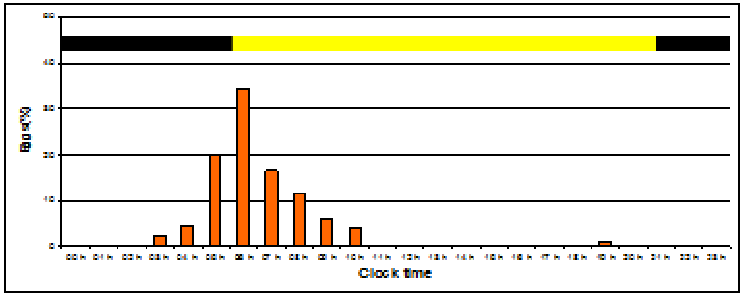


Hen age 23 wks; N=172 eggs; 13 L : 5 D : 3 L : 3 D

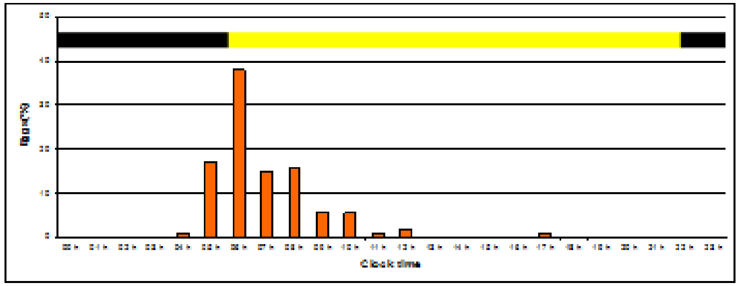


Abrupt Light Increase Treatment

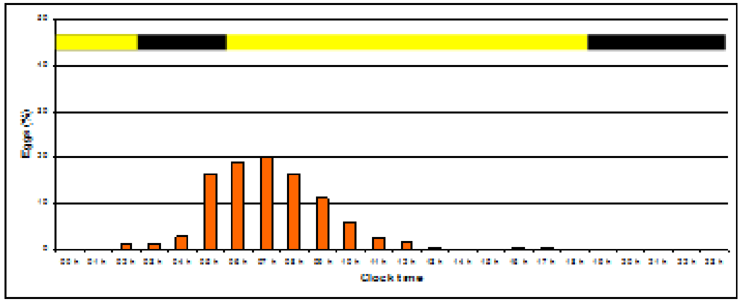
Hen age 21 wks; N=181 eggs; 15 L : 9 D



Hen age 22 wks; N=129 eggs†; 15.5 L : 8.5 D



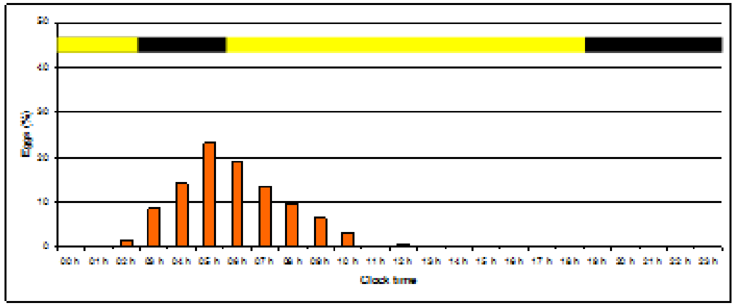
Hen age 23 wks; N=170 eggs; 13 L : 5 D : 3 L : 3 D



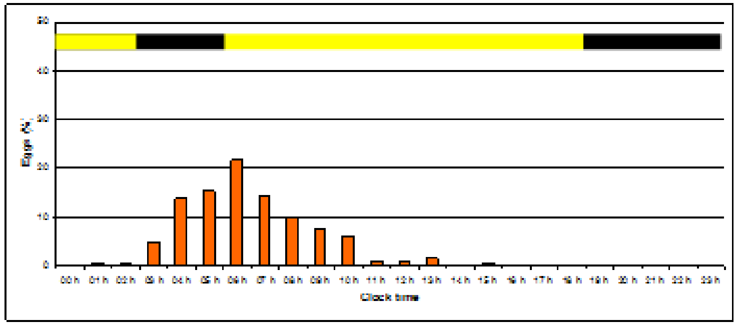
† video system failure reduced data collection in the week

Gradual Light Increase Treatment

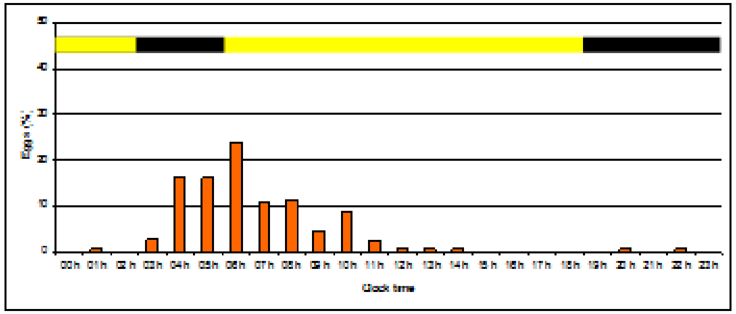
Hen age 24 wks; N=185 eggs; 13 L : 5 D : 3 L : 3 D



Hen age 25 wks; N=180 eggs; 13 L : 5 D : 3 L : 3 D

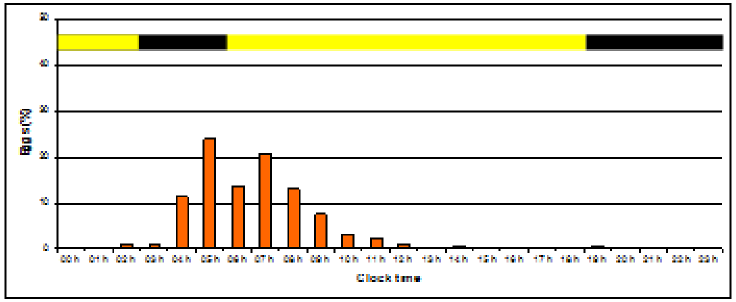


Hen age 26 wks; N=175 eggs; 13 L : 5 D : 3 L : 3 D

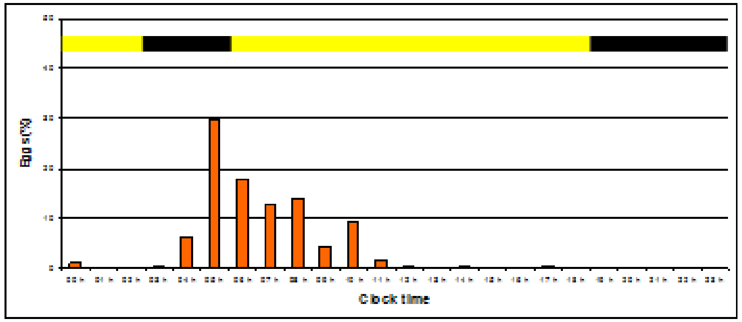


Abrupt Light Increase Treatment

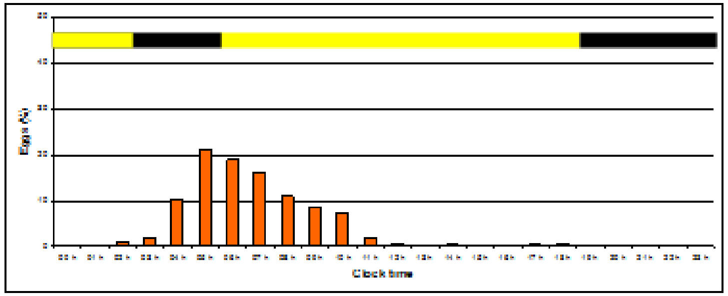
Hen age 24 wks; N=184 eggs; 13 L : 5 D : 3 L : 3 D



Hen age 25 wks; N=178 eggs; 13 L : 5 D : 3 L : 3 D



Hen age 26 wks; N=175 eggs; 13 L : 5 D : 3 L : 3 D



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9 Plain English Compendium Summary

Project Title:	The importance of nests for the welfare of laying hens
AECL Project No.:	VA-01
Researchers:	Greg Cronin and John Barnett
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Email:	g.cronin@usyd.edu.au; john.barnett@unimelb.edu.au
Objectives	To investigate the importance of nests for the welfare of laying hens.
Background	A previous AECL project DAV 197A (Welfare of laying hens in furnished cages) showed that nest boxes, dust baths and perches, when present, were well utilised by laying hens. However, in cages containing a nest box, 62% of eggs were laid in the nest box; thus about one-third of eggs were laid outside the nest box, on the wire cage floor. A major question in the welfare debate for the egg industry concerns whether nest boxes are important to hen welfare.
Research	This project focused on hens' choice of egg-laying site in modern cages and the relationship between egg laying site and stress physiology (as a measure of bird welfare). Using video technology, egg laying patterns and consistency of laying in the nest box were determined. Stress physiology parameters were measured in individual birds and related to egg-laying characteristics.
Outcomes	By about their 10th egg, most hens were consistent in their choice of egg laying site, either in the nest box or on the wire floor. Although more eggs were laid in nest boxes (60-70%) than on the wire floor outside the nest boxes, having a nest box in the cage resulted in increased stress for 23 week old birds, probably due to competition for access at times of peak egg laying. Thus, a nest box in the cage resulted in a short-term increase in the level of stress in young hens. Blocking the entrance to the nest box compared to 'sham' blocking for consistent nest box layers aged 40 weeks, produced a similar stress response over the first 2 days, with minor differences on the third day but no differences in the subsequent 3 weeks. Manipulating the light-dark schedule to induce a proportion of hens to lay in darkness, and thus reduce the use of the nest box, did not adversely affect the welfare of the hens, based on both physiological measures and the quietness of hens.
Implications	More than 80% of Australia's eggs are produced in cage housing systems. Pressure is mounting to ban cages, or at least to introduce cages which include a nest box. However, the presence of a nest box in the cage in fact increased the stress levels in young laying hens for a short period of time, compared to birds in cages without a nest box. Furthermore, there was no evidence of a relationship between nest box use and improved welfare. Thus, the capital expense of incorporating a nest box in cages will not necessarily improve the welfare of laying hens. Some opponents of cage housing believe nest boxes are critical for hen welfare, because birds are highly active before egg laying in the absence of a nest box. However, hens laying in darkness do not perform pre-laying nest-searching behaviour. Presumably, light raises the bird's motivation to seek a nest site and the lower light level inside the nest box de-motivates (or terminates) the searching phase and hens move directly into the sitting phase of egg laying. As most eggs laid by hens in the dark are laid on the wire floor with no impact on stress levels, this suggests that the nest box may not be important to hen welfare, at least when egg laying occurs in the dark.
Publications	Cronin GM, Butler KL, Desnoyers MA, Barnett JL (2005) The use of nest

boxes by hens in cages: what does it mean for welfare? (Proceedings of the 7th European Symposium on Poultry Welfare, Lublin, Poland). Animal Science Papers and Reports, 23 (Supplement 1): 121-133.
Cronin GM, Desnoyers MA (2005) Behavior around oviposition by hens in cages with and without nest boxes. In: Proc. 5th International Conference on Methods and Techniques in Behavioral Research, Wageningen, The Netherlands; 30 August to 2 September, 2005; Eds. MR Ballintijn, CA Bruisten-Jeannot, F Grieco, LWS Loijens, LPJJ Noldus, J Van Santen, PH Zimmerman; pp. 671-674.
Cronin GM (2006) Nesting behaviour of laying hens. Video poster presented that the PIX2006 Conf., Surfers Paradise, 2-4 April 2006.
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